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# All to Valencia Aquaculture Europe 2025



Aquaculture Europe has become the one meeting point for the whole European aquaculture sector for many years now, and this is especially the case for this event in Valencia with its inspiring theme for us all "**Aquaculture for everyone**". No doubt that aquaculture is a key provider of healthy and sustainable seafood worldwide. In Europe, Spain is exemplary on how it has developed a variety of marine and freshwater productions, integrated in their environment, and recognized by society. Yes, aquaculture is for everyone, and everyone has to acknowledge its importance and the multiple services it provides!

Thus, the European Aquaculture Society warmly invites the entire aquaculture community—scientists, industry leaders, policy makers, producers, educators, and students—to Aquaculture Europe 2025, here in Valencia, Spain**.** From cutting-edge technologies to sustainable practices, Aquaculture Europe 2025 will be a vibrant platform to share knowledge, forge partnerships, and explore how aquaculture can meet global food, climate, and societal challenges.

What will you get in Valencia?

- A top-tier scientific program covering all aspects of aquaculture research and innovation, with 40 Scientific sessions covering all disciplines, systems, species – this is more and more exciting from year to year, sometimes even difficult to choose which session attend! We have received more than 840 abstracts, which will be showcased through e-posters and more than 500 oral presentations
- Inspiring plenary sessions by key experts - Carlos Duarte on regenerative aquaculture, Elisabetta Giuffra on leveraging the value of functional genome annotations and Joan Riera on seafood consumption.
- An Industry forum, an Innovation forum, an FAO workshop on its "Guidelines for Sustainable Aquaculture" and a stimulating panel/roundtable organised by the Network for Women in Aquaculture.
- A fantastic international trade show where you will see the latest innovations available on the market
- Field visits to key aquaculture sites and research facilities
- Unique networking opportunities—from expert panels to wonderful tapas evenings!

As always, Aquaculture Europe is not just a conference—it's the meeting point for the entire European and international aquaculture family. Whether you're looking to present research, discover solutions, or shape the future of our blue food systems, AE2025 is where it all comes together. We welcome 2500 attendees from all over the world, including you!

This would not have been possible without be the involvement of the Next Generation Marine Science programme, ThinkInAzul, the Steering committee chaired by Jaume Perez-Sanchez (CSIC), the program co-chairs Diego Mendiola (Caviar Pirinea) and Martin Føre (SINTEF), and the local organizing Committee. They all put in tremendous efforts to make this conference a success, together with the EAS Home Office and our long-standing partners Mario Stael and John Cooksey.

A big thanks also for the support of our local partners, national and regional governments, our Gold Sponsor Skretting, our Silver Sponsors Biorigin, Dibaq, HIPRA, INVE and miXscience, Session Sponsor CTN and our media partners, with a special mention for MisPeces who do such excellent coverage of all our events.

The week will be too short, but so good!

Marc Vandeputte
EAS President

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# ABSTRACTS

# INFECTION WITH MOBILE STAGES OF SALMON LICE AND CALIGUS ELONGATUS IN A SALMON FARM NETWORK

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In Atlantic salmon aquaculture, understanding the infection dynamics of sea lice is critical for sustainable management. For salmon lice *(Lepeophtheirus salmonis*) the most common infection pathway is direct infection through the planktonic copepodite stage. However, the high level of handling of the farmed fish where a portion of the mobile lice fall off might induce an indirect infection pathway where mobile lice survive and successfully find a new host. The other sea lice species that affects farmed salmon in the north Atlantic is the generalist *Caligus elongatus* which has been found on more than 80 fish species. This species has different behavioural traits than the salmon lice and the relative contribution of direct and indirect infection pathways to the overall infection pressure on farmed fish is not well understood.

In this study indirect and direct infection pathways of salmon lice and *C. elongatus* were investigated by analysing comprehensive sea lice monitoring data from the Faroese salmon farming industry, covering more than 1200 cage deployments. Indirect infection was identified when mobile or adult lice were found on newly deployed fish before development from a copepodite was possible according to temperature-based development models for the two species.

This approach provided a detailed examination of indirect infection dynamics in an operational farming environment. Preliminary observations suggest a notable difference between the two lice spices, with indirect infection substantially more prominent for *C. elongatus* than salmon lice.

# SOCIAL ACCEPTANCE OF BALTIC SEA BLUE MUSSELS

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**Introduction**
The Baltic MUPPETS project is an investment in a blue bioeconomy that aims to deliver economically and environmentally viable businesses with a positive socio-economic impact. The project sets out to create an entirely new value chain based on small blue mussels, enabling a new circular economy in the Baltic Sea region and supporting mussel farmers throughout Europe to develop, diversify and scale their existing businesses.

The development of sustainable aquaculture is a current political issue and it is considered a future industry with great potential. Production of aquaculture-based products such as mussels is also labor-intensive and can therefore contribute to increased employment rates, especially in sparsely populated areas. A fully developed mussel production could, for example, create complementary jobs for local fishermen, but also facilitate changes of ownership and new recruitment through the expansion, diversification and long-term profitability. In addition, depending on which end product that is developed, a number of other industries/occupational categories can be activated. For instance, within fishing or food tourism.

Through cultivation, harvesting and efficient marketing, the Baltic blue mussel can thus help to return nutrients from sea to land and at the same time transform access nutrients into a sustainable job-creating natural resource. Put in another way, mussel farming could provide a way to integrate environmental objectives with economic and socio-economic goals in order to advance and strengthen the three interdependent pillars of sustainable development.

Using three case studies (ECOPELAG in Sweden, Wittrup Seafood in Denmark and Kieler Meeresfarm in Germany), the business cases are explored, including bottlenecks such as legislative and social acceptance.

**Site-selection and technology for social acceptance**
When establishing a mussel farm it is important to consider the public view when selecting the site and technology. All farmers in Baltic MUPPETS use submerged farming solutions, meaning the infrastructure is also less visible over the surface. This can increase the acceptance. Similarly, it is important to choose sites that inflict as little as possible with the public, both ecstatically and recreationally, as well as other industries.

**Working with the public**
It is important to early gain the approval of the public when establishing a mussel farm. Inclusion in planning processes, site-selection, etc. can evoke a feeling of influence and lower negative perception.

The public perception of mussel farms has been a challenge for some mussel farmers in Denmark. The BONUS Optimus project showed the Danish perception was generally more negative than the German one. This might be due to more experience as mussel farming is not equally established in Germany. Further, there has been much debate about mussel farming and fishing in the media. However, this is recently changing as the political circumstances are promoting mussel farming more whilst banning trawling. In Kiel, Kieler Meeresfarm has worked a lot with public engagement in the farms, and putting a lot of effort into education. Locals can “adopt a line”, giving some ownership and increasing acceptance. Also, working together with leisure boat associations to highlight seasonal opportunities for collaboration highlights the non-competitive nature of the farm and adjacent industries. In eastern Sweden, ECOPELAG is working closely with the municipality to gain approval by the public, e.g. by cooperating with the local “Nature Room”, a public nature education initiative. Here they mainly focus on teaching about the benefits the farm can give to the local environment and biodiversity.

**Bottlenecks for social acceptance**
A bottleneck for the farming of mussels in the Baltic Sea is the general scepticism of the water quality and product safety. Without approval of the products, the business models are not sustainable as the demand is reduced. For this, it is important to be transparent about quality testing and assurance. This can be difficult depending on the legal testing requirements, and too rigorous or unsuitable processes can hinder quick communication about quality, e.g. if tests need to be done in certain laboratories and waiting times are long.

# AUTOMATED INDIVIDUAL FEEDING INTAKE QUANTIFICATION IN EUROPEAN SEABASS (*Dicentrarchus labrax*) USING MULTI-MODEL COMPUTER VISION AND FISH RE-IDENTIFICATION

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**Background and Objectives**

Precision feeding in aquaculture requires accurate monitoring of individual fish feeding behavior to optimize feed conversion ratios and reduce environmental impact. Traditional methods for assessing feeding intake in European seabass (*Dicentrarchus labrax*) rely on individual compartments that induce stress or bulk measurements that fail to capture individual variations critical for selective breeding and personalized nutrition strategies. This study presents a novel automated system for real-time individual feeding intake quantification using advanced computer vision and fish re-identification technologies.

**Methods**

We developed a comprehensive two-pass computer vision pipeline integrating four specialized YOLO models: pellet detection, open mouth detection, fish eye tracking, and fish face identification. All of the models are built on yolo11n except the fish face detection which use a yolo11n-obb. Manual annotations are done with Label Studio 1.16.0 and trained on NVIDIA A40 GPU provided by MARBEC GPU. The system employs a custom CNN-based re-identification network trained on fish-specific embeddings to maintain individual fish identity across video frames (Figure 1.). The whole pipeline include: (1) pellet disappearance tracking with configurable temporal windows, (2) proximity-based feeding event detection linking pellet consumption to open mouth behavior, (3) persistent fish tracking using eye detection as anchor points, and (4) dynamic conflict resolution for accurate individual attribution. The system processes video data through a first pass for global fish identity establishment, followed by a second pass for real-time feeding event detection and attribution.

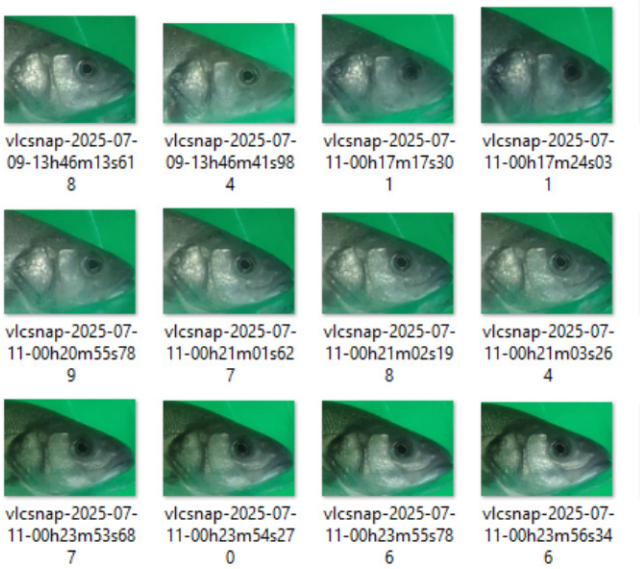


**Figure 1.** Examples of training dataset for fish re-identification

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## Results

Testing on European seabass feeding trials demonstrated robust performance in individual fish tracking and feeding event detection (Figure 2). The system successfully tracked multiple fish simultaneously, maintaining individual identities. Feeding events were accurately detected by correlating pellet disappearance (tracked over minimum 5 frames) with open mouth detection within configurable proximity thresholds (200 pixels). The re-identification network achieved reliable fish recognition using normalized embeddings with Euclidean distance metrics, enabling consistent individual attribution across extended observation periods.

## Discussions and future plan

This automated system represents a significant advancement in precision aquaculture. Currently, there are several errors including too many unknown fish and pellet and eye detection confusion. We are trying to improve the identification by using a deeper CNN architecture and refine the training dataset. We will try also to run this technology on a longer video. We are also developing a whole-body identification and key frame feeding event detection.

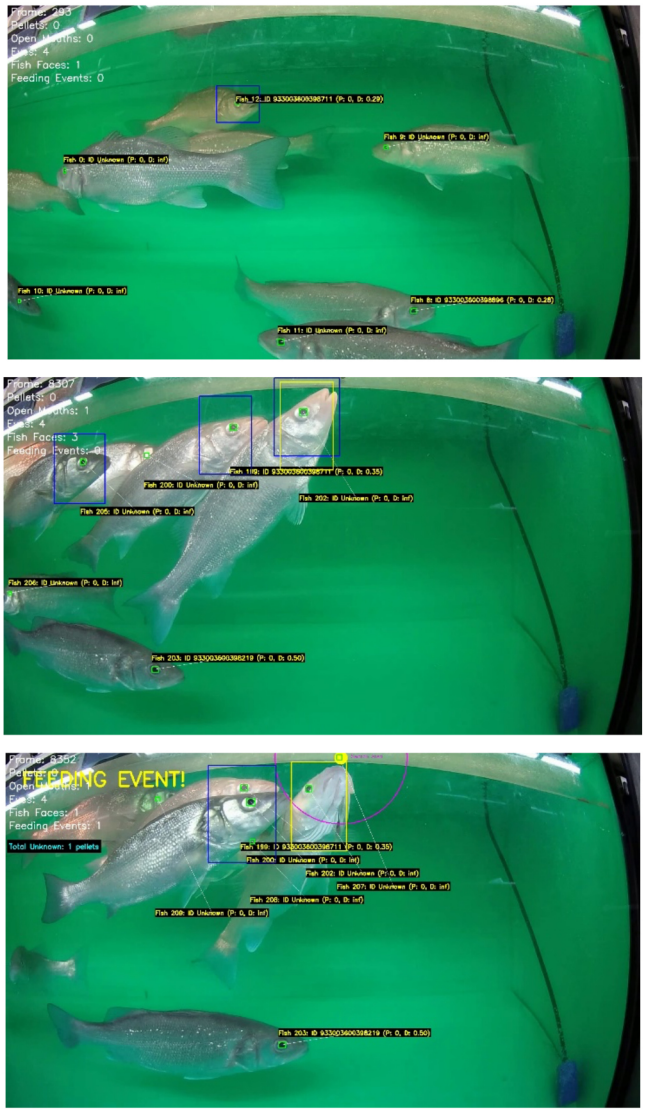


**Figure 2.** Screenshots of the output video. Blue box represents the fish face detection who feed the re-identification model. Yellow box represents the open mouth detection. Green box is the eye tracker with a white line to the tracker id (fish+numer), real ID, P which is the pellet eaten and D which is the Euclidean distance for identification. Feeding event are triggered by the disappearance of tracked pellet and the presence of open mouth in the search area (magenta circle). The feeding event then attributed to the nearest tracked eye

# PROTEOMIC INSIGHTS INTO TAURINE-INDUCED CHANGES ON EUROPEAN SEA BASS MYOTUBES

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**Introduction**

European sea bass (*Dicentrarchus labrax*) is one of the most commercially valuable species in the Mediterranean aquaculture. However, the use of highly substituted diets for European sea bass is hampered due to the high carnivorous habits of this species. The non-protein amino acid, taurine, has been previously reported as a potential dietary supplement in fish aquaculture, enhancing both growth and antioxidant capacity (D. Montero et al., 2023; P. Salze and Allen Davis, 2015). Here, to better understand the signalling response triggered by taurine in muscle, proteomic and phosphoproteomic analyses were carried out on cultured myotubes from European sea bass.

**Methodology**

European sea bass juveniles (10 ± 2 g of body weight) provided by Culmarex (Mallorca, Spain) were maintained in our indoor facilities at the University of Barcelona. Muscle cells were extracted from 11-12g of white epaxial muscle from juveniles and cultured in quadruplicate (n=4) for 13 days until myotube development stage (García-Pérez et al., *under review*). Cells were treated 24h with 10mM taurine (showed no effects on cell viability) and protein extracted with 8M urea buffer containing a mix of proteases and phosphatases inhibitors. Samples were reduced, alkylated, and digested with LysC and trypsin. Digested peptides were desalted with reverse phase C18 columns, keeping an aliquot for proteome analysis and using another aliquot for phosphopeptide enrichment by IMAC using Fe-NTA magnetic beads. Data acquisition was performed by nano LC-MS/MS using Evosep One chromatograph coupled to an Orbitrap Eclipse mass spectrometer with DDA methods. MaxQuant software was used to identify and quantify the proteome and phosphoproteome using European sea bass database from Uniprot, whereas Perseus and R software were used for bioinformatic analyses.

**Results and discussion**

We quantified 2643 proteins, of which 267 (10.1%) showed significant changes in abundance between taurine and control groups. Hierarchical cluster analysis of z-score normalized changes in proteome abundance allowed to discriminate between control and treatment groups (Fig.1a). Downregulated proteins upon taurine treatment were enriched in gene ontology biological process terms (GOBP) related to cell development (Fig.1b), while mostly protein translation processes were upregulated. We identified 7259 phosphosites, of which 1179 (16,2%) displayed significant differences in intensities between the two groups (Fig.2). Several targets of the mTOR signalling pathway showed increased phosphorylation upon taurine treatment, such as AKT, 4EBP1, and EIF4EBP1, as well as transcriptional factors (e.g., EIF3A) involved in protein synthesis. These findings support a proteinogenic stimulus of taurine in muscle cells by enhancing mTOR signalling and ribosomal activity, thus indicating its potential as a dietary additive for stimulating muscle growth in European sea bass.

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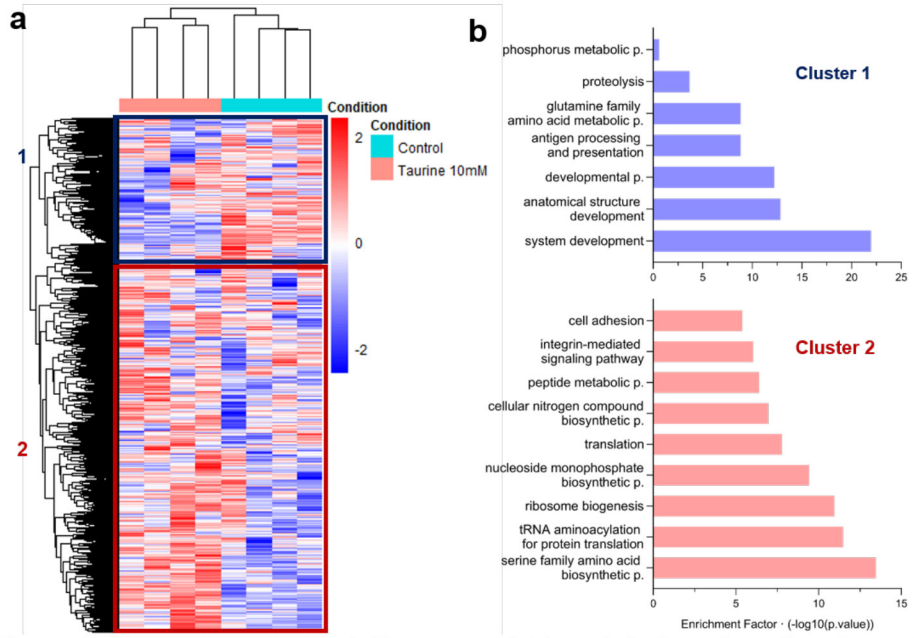


**Figure 1**: Proteomic analysis. a) Heatmap and hierarchal clustering of total proteins detected. b) Significantly enriched GOBP terms from main clusters of heatmap.

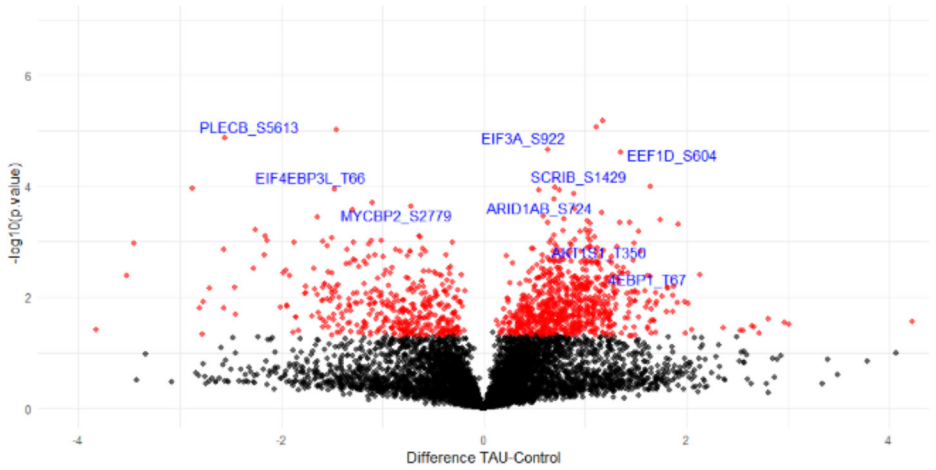


**Figure 2**: Volcano plot comparing taurine and control group from phosphoproteomic data. (n=3, t-test p-value < 0.05). Highly significant phosphosites involved in mTOR signalling are indicated.

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# HOW INPUT DATA QUALITY AFFECTS AQUACULTURE ZONING

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**Introduction**

In the framework of the PLASMAR (2017-2021) and PLASMAR+ (2021-2023) projects, we developed a multi-parameter spatial analysis to assess the suitability of marine areas for aquaculture development in the Canary Islands and Madeira archipelago. Zoning analyses integrated diverse criteria structured as marine environmental conditions, conservation targets, oceanographic potential, coastal uses, and maritime operational sectors. The results have been presented on Aquaculture Europe 2021 and directly applied to inform the Maritime Spatial Planning (MSP) processes in both regions, supporting evidence-based decisions for aquaculture site allocation.

**Methodology & results**

Following, the ReMAP project (2022-2025) applied the MSP Data Framework (Technical Expert Group on Data for MSP, 2023) to develop an Input Data Module. This tool allows for a systematic evaluation of the spatial data underpinning MSP, focusing on thematic availability, transparency, quality, coverage, and resolution. We applied the module to assess the input datasets used in the aquaculture suitability analyses for Madeira and the Canary Islands. The findings reveal how variations in data quality, completeness and coverage can significantly influence the outcomes of spatial planning and, ultimately, the identification of suitable areas for aquaculture.

**Conclusion**

This is the first analysis of its kind that shifts attention to how we *feed* the model—examining the input data itself. While discussions around spatial models often focus on their internal mechanisms or just on delivered "model" results we rarely scrutinize the type and quality of data entering the model and how that input shapes the final results. This approach brings a new level of transparency and critical understanding to the modelling process in maritime spatial planning.

As one of 10 ReMAP modules, developed for MSP evaluation and monitoring, the Input Data Module is as an open-access, open-source tool. It is easy to use, designed as an intuitive auto-assessment system that simplifies the evaluation process while decreasing complexity and increasing reproducibility. The tool is immediately available to the aquaculture and MSP community for direct use, adaptation, and integration into planning processes following this presentation.

# NUTRIENTS' DIGESTIBILITY AND INTESTINAL MORPHOLOGY OF EUROPEAN SEABASS AND GILTHEAD SEABREAM FED DIETS WITH BLACK SOLDIER FLY OIL UNDER TWO REARING TEMPERATURES

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**Introduction**

The growing demand for high-quality seafood – driven by population growth and increasing consumer awareness of the health benefits of fish consumption – is propelling aquaculture expansion, which in turn increase the need for aquafeed and sustainable raw materials. Black soldier fly larvae oil (*Hermetia illucens*, BSFLO), rich in lauric acid (C12:0; 30-70%), a medium-chain saturated fatty acid (SFA), is gaining attention for its potential functional properties. However, water temperature can significantly affect the digestion and absorption of dietary lipids in fish. This is particularly relevant for lipid sources rich in SFA, which generally have higher melting points than their unsaturated counterparts of the same chain length. As a result, temperature-dependent changes in the physicochemical properties of dietary lipids may influence their bioavailability and utilization by fish in cold water conditions. Therefore, this study aimed to explore the impact of the dietary BSFLO inclusion on macronutrient and fatty acid digestibility, as well as intestinal morphology, of European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) – two key species in Mediterranean aquaculture – reared at 15 °C and 23 °C, to simulate average seasonal conditions (winter and summer) in the Mediterranean.

**Material and methods**

A control diet (CTRL) was formulated with 6% fish oil and 5% poultry oil (PO). Three other experimental diets were formulated to include 1.7%, 3.4% and 5.2% of BSFLO (Entrogreen® - Ingredient Odissey S.A), at the expense of PO (BSFLO1.7, BSFLO3.4 and BSFLO5.2, respectively). All experimental diets contained 1% $Cr_2O_3$ as an inert marker. These diets were tested in triplicate groups of seabass and seabream, maintained under two temperature conditions (15 °C and 23 °C) in a recirculating aquaculture system (RAS) with sedimentation columns for faeces collection. Fish were fed to apparent satiation three times daily and faeces were collected daily and stored at -80 ºC. After freeze-drying, faecal chromium, crude protein, total lipids, crude energy, and fatty acids levels were determined, and their apparent digestibility coefficients (ADC) were calculated. In addition, at the end of digestibility trials, a histomorphometric evaluation of the fish anterior intestine was performed.

**Results**

Dietary inclusion of BSFLO did not impact the anterior intestinal histomorphology of either seabass or seabream. However, water temperature significantly influenced European seabass cross-sectional-area and -perimeter, as well as the thickness of the muscularis, submucosa, and lamina propria, villus length and the total number of acid goblet cells, which were significantly reduced at the lower temperature. These intestinal changes were associated with a slight reduction in protein digestibility from 91% at 23 ºC to 90% at 15ºC. Total lipid ADC values remained similar between temperatures, but the digestibility of SFA decreased at 15 °C (93% vs. 98%), resulting in lower energy ADC values (81% at 15 ºC vs. 83% at 23ºC). BSFLO dietary inclusion had no impact on seabass macronutrient, fatty acid, or energy digestibility, despite the higher SFA content in BSFLO diets. In contrast, in seabream, the digestibility of SFA was significantly higher in fish fed BSFLO3.4 and BSFLO5.2 diets than those fed the CTRL diet (95% vs. 97%), regardless of water temperature. Macronutrients, fatty acids and energy digestibility remained similar at 15ºC and 23ºC. Likewise, temperature had a minimal effect on the intestinal structure, with the only changes observed being a reduction in thickness of the muscularis and a lower number of acid goblet cells at 15ºC.

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**Discussion and conclusion**

The results of this study highlight species-specific responses to dietary BSFLO and water temperature in European seabass and gilthead seabream. While BSFLO inclusion had no significant effects on intestinal histomorphology or nutrient digestibility in either species, water temperature played a more critical role in seabass, where rearing lower temperatures (15 °C) led to structural intestinal changes and a slight reduction in protein and SFA digestibility. In contrast, seabream exhibited a similar digestive function at 15ºC and 23ºC and even furthered from improved SFA digestibility with BSFLO dietary inclusion. These findings suggest that BSFLO is a viable lipid source for seabream and can be effectively used in aquafeeds without compromising nutrient absorption. However, long-term pilot studies are needed to validate these results in seabream and assess the physiological consequences observed in seabass, namely the potential impact on growth performance and fish health. In addition, complementing digestibility and histological evaluations with other types of analyses, such as digestive enzyme activity or gene expression profiling, would help to better understand the mechanisms underlying these species-specific responses.

**Acknowledgments**

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# BEYOND THE SPAWN: EPIGENETIC AND TRANSCRIPTOMIC FOOTPRINTS OF SEASONAL MANIPULATION IN ATLANTIC SALMON FRY

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## Introduction

Manipulating spawning time doesn't just extend the breeding calendar - it initiates molecular responses that can shape the next generation. In aquaculture, altering environmental cues such as temperature and photoperiod to shift the spawning window is a well-established strategy to ensure year-round offspring production. While effective in practice, the broader biological consequences of this approach across generations remain insufficiently understood. Delaying spawning in Atlantic salmon reduced egg quality but had no effect on fecundity and improved fry size (Fjelldal et al. 2024). Manipulating spawning time also altered egg nutrient profiles, which may suggest potential impacts on offspring through metabolic programming (Skjærven et al. 2020; Skjærven et al. 2022). Saito et al. (2025) linked a manipulation of spawning time to altered DNA methylation profiles in salmon fry livers with potential implications for growth and development, however, little is known about how such reproductive interventions impact muscle growth. This study explores how extending the spawning season of Atlantic salmon - comparing delayed vs. normal spawning - impacts the nutritional, transcriptomic and epigenetic programming of both muscle and liver in first-feeding fry.

## Material and methods

Unfertilized eggs and first-feeding fry (~845 degree-days) derived from broodstock exposed to either normal or delayed spawning conditions were sampled. We analysed free and protein-bound amino acid profiles in unfertilized eggs and first-feeding fry to assess nutrient status and early metabolic regulation. RNA was extracted from muscle and liver of first-feeding fry for RNA-sequencing and genomic DNA for EM-sequencing to assess gene expression and methylation landscapes.

## Results

Preliminary results revealed a stronger transcriptional response in muscle than in liver of first-feeding fry, with an approximately fivefold increase in differentially expressed genes between the spawning groups, indicating a more pronounced tissue-specific response in muscle at this early developmental stage. In parallel, amino acid profiling of both eggs and fry showed distinct metabolic signatures between the spawning seasons, which are suggestive of compensatory adjustments in response to the nutrients supplied by the mother to the yolk. These metabolic shifts showed alignment with the gene expression changes. Across both tissues, differentially expressed genes were enriched in pathways related to energy metabolism, protein turnover, and one-carbon (1C) metabolism - key processes known to influence the methylation potential. Ongoing epigenetic analyses will provide insight into regulatory pathways affected by these molecular changes at early life stages.

## Discussion and Conclusion

These findings indicate that extending the spawning season modulates offspring development at both metabolic and molecular levels. Notably, delayed spawning resulted in larger fry, suggesting enhanced early growth potential despite reduced egg quality. This growth advantage may be linked to the transcriptional changes observed in muscle tissue, which appears particularly responsive to altered spawning conditions. By combining gene expression, amino acid profiles, and epigenetic data, this study provides a foundation for understanding how altered spawning timing shapes offspring physiology at multiple biological levels. This knowledge may support the refinement of spawning strategies and nutritional protocols to optimize robustness performance in commercial aquaculture.

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# MEASURING IMMUNE RESPONSES AND PATHOGEN DETECTION TO SUPPORT FISH DISEASE DIAGNOSIS ACROSS SPECIES

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**Introduction**
Monitoring immune responses in non-lethal epithelial tissue samples, combined with pathogen detection in environmental samples, is a powerful approach for diagnosing mucosal diseases in fish. This approach is particularly valuable in aquaculture and wildlife contexts, where sacrificing animals for testing is not feasible. We applied and evaluated this strategy in several case studies, including multifactorial gill disease in common carp (Cyprinus carpio) and gill disease in rainbow trout (Oncorhynchus mykiss), based on both diagnostic submissions and selected infection experiments. Other case studies included hyperpigmentation in common dab (Limanda limanda) and Fancy-Tailed Guppy Virus (FTGV) infection in guppies (Poecilia reticulata). Our aim was to assess the diagnostic value of epithelial immune profiling alongside the molecular detection of pathogens in both fish and their environment.

**Methodology**
Samples included gill biopsies, skin/gill swabs and water collected during natural outbreaks and experimental infections. These samples were stored at −20 °C prior to analysis. Pathogens were concentrated from the water samples using various filtration methods, and then detected via qPCR/RT-qPCR. Host immune responses were profiled using RT-qPCR or Fluidigm arrays and correlated with pathogen load and histopathological changes. In dab, hyperpigmented and normally pigmented skin samples from the same fish were compared using 16S metabarcoding and immune marker profiling. In guppies, samples from outbreak-associated and laboratory-raised populations were analysed for FTGV using virological and molecular assays, metabarcoding and immune profiling.

**Results**
Epithelial immune profiling revealed pathogen-class-specific signatures, with antiviral responses observed in viral gill diseases and proinflammatory profiles in bacterial infections. Gill swabs proved to be as reliable as biopsies for detecting these signatures. Water filtration at 0.20–0.45 µm enabled the sensitive detection of gill disease pathogens, often at the earliest stages of infection, and pathogen nucleic acids persisted in the environment after the removal of infected fish. In dab, however, no microbiome differences or antiviral responses were detected between hyperpigmented and normal skin, suggesting a non-infectious cause. FTGV was detected for the first time in Europe in outbreak-associated guppy populations. Partial genome sequencing revealed significant viral diversity; however, immune profiling was uninformative due to late, subclinical infection stages.

**Conclusions**
Measuring immune responses in epithelial tissues alongside environmental pathogen detection enables the early, non-lethal diagnosis of mucosal diseases in diverse fish species. This integrated approach can be used for differential diagnosis (e.g. viral versus bacterial gill diseases), surveillance (e.g. FTGV in guppies) and outbreak management, particularly in settings where post-mortem sampling is impractical. Our findings emphasise the effectiveness of combining immune profiling with environmental DNA/RNA detection for improving fish health monitoring in aquaculture and wild populations.

# USING THE LARVAL STAGES OF ATLANTIC SALMON AND ZEBRAFISH TO STUDY THE ANTIVIRAL AND IMMUNOMODULATORY EFFECTS OF 25-HYDROXYCHOLESTEROL (25HC) AGAINST TILAPIA LAKE VIRUS (TILV)

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## Introduction

25-hydroxycholesterol (25HC) is an oxidised derivative of cholesterol that can be produced either enzymatically, via the action of cholesterol 25-hydroxylase (Ch25h), or through non-enzymatic pathways involving reactive oxygen species (ROS). In mammals, 25HC has been associated with the inhibition of viral entry into host cells, indicating a potential role in immune defence. However, its impact on antiviral responses in fish remains unclear. This study aims to investigate the influence of 25HC on viral infections and the immune response in the larval stages of zebrafish and Atlantic salmon, which are relevant to aquaculture, to demonstrate their potential in the search for new antiviral or immunomodulatory substances.

## Methodology

Atlantic salmon and zebrafish larvae were injected with 25HC into the pericardium, and two hours later were infected with Tilapia Lake Virus (TiLV). Additionally, we developed a new zebrafish knockout line with a five-base-pair deletion in the gene encoding cholesterol 25-hydroxylases (ch25hb-/-), using CRISPR/Cas9 gene editing. Larvae from both the ch25hb-/- and the ch25hb+/+ lines were infected with TiLV by intrapericardial injection. Viral load and the expression of immune-related genes were studied by RT-qPCR.

## Results

A significantly lower viral load was observed in Atlantic salmon and zebrafish larvae treated with 25HC compared to the control group. This suggests that 25HC treatment reduces viral entry and/or replication upon infection. Furthermore, we found that the expression of immune-related genes encoding proteins involved in the type I IFN pathway (e.g. *rig-I, irf3, irf7, mxa, isg15*) and pro-inflammatory cytokines (*il-1b, il-6*) as well as *ch25hb* was up-regulated in TiLV-infected fish, although almost no significant effect of 25HC treatment was recorded. In case of *ch25hb*$^{-/-}$ knock-out zebrafish, no notable differences in terms of development and survival were observed as compared to the *ch25hb*$^{+/+}$. Interestingly, upon TiLV infection, viral load was significantly higher in *ch25hb*$^{-/-}$ zebrafish compared to *ch25hb*$^{+/+}$ controls, which suggest that loss of *ch25hb* compromises the host's capacity to control TiLV replication. This observation was reinforced by gene expression analysis, which revealed a significant lower expression of multiple antiviral response genes in the *ch25hb*$^{-/-}$ zebrafish.

## Conclusions

This study provides new insights into the function of 25HC during viral infections in Atlantic salmon and zebrafish larvae. Pre-treatment with 25HC significantly reduced viral load in both fish species examined. Additionally, disruption of the ch25hb gene impaired the host's ability to control TiLV infection, highlighting the gene's role in antiviral defence. This shows that zebrafish can be used as a proxy for aquaculture-relevant species. Furthermore, Atlantic salmon larvae can be used to test the antiviral activity of substances in a similar capacity to zebrafish.

# SYSTEMIC DYSBIOSIS AS A CONSEQUENCE OF CARP EDEMA VIRUS INFECTION

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**Introduction**
Carp edema virus (CEV) is a highly contagious pathogen affecting common carp (*Cyprinus carpio*). The virus causes koi sleepy disease (KSD), which is characterized by disturbances of the gill function, resulting in hypoxia, hyponatremia, and hyperammonemia. Our previous studies shown that CEV infection led to immunomodulation and activation of stress response. In the present study, we analyzed the impact of CEV infection on the gill, foregut and hindgut microbiota of the common carp.

**Methodology**
Gill and gut microbiota was analyzed by metabarcoding of bacteria 16S rRNA. Pathomorphological changes of the gill and gut tissues caused by CEV infection were assessed using HE staining. Expression of selected genes encoding proteins involved in antiviral, inflammatory and mucosal response as well as tight junction proteins was analyzed in studied tissues by RT-qPCR. Cortisol and glucose level was measured in blood plasma using ELISA and glucometer, respectively.

**Results**
Infected fish had mean $3.53x10^5$ viral DNA copies in gills, $1.4x10^2$ DNA copies in foregut and $1.49x10^3$ DNA copies in hindgut at 6 day post exposure. Infection induced increase in blood cortisol and glucose levels. Histopathological analysis of the gills demonstrated nearly complete occlusion of the intralamellar space with the accumulation of cellular debris and an infiltration of eosinophilic cells in CEV-infected fish. In gut only minor histopathological changes were recorded. CEV infection clearly impacted gill microbiota, resulting in increased number of bacteria, e.g. *Flavobacterium, Cetobacterium, Aeromonas, Pseudomonas.* In two studied parts of the intestines only in hindgut significant increase of bacteria genera (*Flavobacterium, Cetobacterium* and *Aeromonas*) was observed. Gene expression analysis revealed up-regulation of the expression of anti-viral *mxa, vig 1* and pro-inflammatory *il-1b* in gills, foregut and hindgut upon CEV infection. Among mucosal related genes, only *muc 5b* was up-regulated in gills of infected fish, while in case of genes encoding tight junction protein, expression of *cldn 7* was up-regulated in all studied organs, and expression of *cldn 23* and *cldn 30* was up-regulated in foregut and hindgut.

**Conclusions**
CEV infection induces stress and immune responses and ultimately disrupts microbiota by favoring bacterial growth, paving the way for secondary bacterial infection. Our study helps to understand the effect of CEV infection on the host microbiota and partly explains the mechanisms for occurrence of secondary bacterial infections related to KSD.

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# FIRST PARASITOLOGICAL ASSESSMENT OF FARMED NILE TILAPIA (*Oreochromis niloticus*) IN ANGOLA: PREVALENCE AND DISTRIBUTION IN HUAMBO PRODUCTION UNITS

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**Introduction**

Tilapia (*Oreochromis niloticus*) is a cichlid native to Africa and the Middle East, produced almost worldwide, with 12 species of commercial importance to date. Its global production has grown over the years, reaching a mark of 6.95 million tons in 2024 (FAO 2024; O Presente Rural 2025). In Angola, parasites in tilapia and the diseases they cause have not been studied to date. However, with the widespread and intensified tilapia farming throughout the country and increased consumption, research on the parasitic fauna of tilapia produced in Angola is necessary as a basis for controlling parasite populations, preventing the occurrence of parasitic diseases, and producing safe food. This is a pioneering study in the detection, identification, and assessment of parasite load in tilapia farming in Angola. It contributes to raising awareness about fish health management among producers and aquaculture potential, service providers, and researchers, strengthening the framework for sustainable production.

**Materials and Methods**

A total of 241 specimens of Nile tilapia, clinically healthy, including fingerlings, juveniles, and adults, were randomly collected during September 2024–January 2025 and April–July 2025 in five private tilapia production units (named A, B, C, D & E) of Huambo, Angola, for parasitological analysis, following the sampling procedures described by Ishikawa *et al.,* 2016. The fish were analysed for the detection of ecto and endoparasites using both macroscopic and microscopic methods. Externally, the skin, fins, mouth, eyes, operculum, and gills were examined with the naked eye. Internally, the heart, muscles, gallbladder, stomach, and intestines were inspected. After the samples were collected and the prepared slides were analyzed microscopically (Guzman *et al.,* 1988; Menezes 2000; Mitiku 2021; Pinacho-Pinacho *et al.,* 2023).

**Results**

From the 241 fish examined, 61 were infested with one or multiple parasites belonging to different genera. Different types of parasites were detected, both in internal organs and on the external surface of the body. The most prevalent parasite group was Monogenea (represented by the genera Gyrodactylus, Dactylogyrus, and Cichlidogyrus), with a prevalence of 11.2% detected in the skin and gills. This was followed by Digenea, with 9.5% detected in the intestine. The least prevalent group was Nematoda (larvae and eggs), with 4.6% detected in the intestine and gallbladder. (Table 1).

Table 1. Groups of parasites detected, infested organs and respective prevalence in five fish farms (named A, B, C, D and E) studied.

| Group | EF | PF | P (%) | Organs | Fish farm |
|---|---|---|---|---|---|
| Monogeneans | 241 | 27 | 11.2 | Gills, Skin | A, B, C, D & E |
| Digeneans | 241 | 23 | 9.5 | Gills, Skin | A, B, C, D & E |
| Nematode | 241 | 11 | 4.6 | Intestines, gallbladder | B, C, D & E |
| Total | 241 | 61 | 25.31 | | |

Examined fish (EF), Parasited fish (PF), Prevalence (P).

*(Continued on next page)*

**Conclusion**

This study represents the first parasitological characterization of farmed tilapia in Angola, contributing significantly to the understanding of fish health in the country. The analysis of 241 clinically healthy specimens revealed an overall parasite prevalence of 25.3%, with the highest incidence of monogeneans (11.2%), followed by digeneans (9.5%) and nematodes (4.6%) found in external and internal organs.

The presence of multiple parasite groups across all production units evaluated suggests a widespread and cross-cutting dissemination of these agents in tilapia farming systems in the Huambo region. These findings underscore the urgent need to implement regular health monitoring and sanitary management measures to prevent outbreaks of parasitic diseases that could compromise animal welfare, productivity, and food safety.

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# SPATIAL DISTRIBUTION OF CULTURED JUVENILES OF *Hediste diversicolor*

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**Summary**
The development of intensive cultivation of *H. diversicolor* requires the production of quality juveniles in adequate quantities for fattening. Considering the spontaneous and asynchronous reproduction of this species in captivity that produces successive batches of juveniles and the confinement of juvenile specimens in the top centimeters of the sediment, this experiment evaluates if the juvenile's distribution in the breeding tanks is homogeneous or it has a heterogeneous distribution with an aggregation pattern for application in the development of management protocols.

**Introduction**
The cultivation of polychaetes has been gaining interest due to their multiple applications; such as individual commercialization as bait for sport fishing, raw material for aquaculture feeds replacing fish oils and meals, or as direct food to stimulate reproduction in the shrimp farming industry, among others (Rasines et al. 2023). Under cultivation conditions, with sufficient food supply, *Hediste diversicolor* reproduces spontaneously and asynchronously, providing successive generations of juveniles. The juveniles are confined to the upper few centimeters of the sediment (Davey, 1994). Their distribution may affect the estimation of juvenile production and impact subsequent management, including the removal of juveniles from the breeding and independent fattening tanks. To advance the development of a fattening protocol for this species, this study analyzes the distribution of juveniles during three reproductive events in 1 m² cultivation tanks of *Hediste diversicolor* at the specific experimental polychaete cultivation research facilities (PAU) of the Spanish Institute of Oceanography-CSIC.

**Materials and Methods**
To estimate juvenile distribution, three 1 m² tanks were seeded with 20 cm of sand and approximately 15 cm of water column, using reproductive specimens cultivated at our facilities (P1 = 338 individuals, mean wet weight (MWW) = 1094 mg; P2 = 338 individuals, MWW = 1340 mg; and P3 = 300 individuals, MWW = 1024 mg). Cultivation was carried out in an open water circuit under natural temperature and salinity conditions, and photoperiod 16L:8D, feeding with commercial sole feed at a rate of 4% of the worm biomass per day, adjusting as needed to ensure availability.

One and a half months after seeding the breeders, a sampling was conducted to detect and quantify the juveniles produced. The sampling consisted of siphoning the top 2 cm of sand from alternating 10 x 10 cm quadrats, sampling every other square across the tank surface, resulting in 50 samples per tank. These sand and water samples were homogenized by gentle mixing, and three 5 ml subsamples were examined under a stereo microscope to identify juveniles of the species. The unsampled quadrats were estimated by calculating the mean number of juveniles in the adjacent sampled quadrats. The result was three 10 x 10 data matrices with values of juvenile identified per quadrat.

To assess the degree of homogeneity in the juvenile production tanks, Moran's I indices and associated p-values for each data matrix were calculated by permutations to evaluate the significance of the index. Additionally, coefficients of variation for each matrix were calculated.

**Results and Discussion**
A total of 20671, 13171, and 1208 juveniles were obtained in tanks P1, P2, and P3, respectively. The smallest juveniles detected had 8 setigerous segments, and the largest had 13. The distribution of juveniles identified in the subsamples and estimated in each tank is shown in figure 1.

*(Continued on next page)*

**P1**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 11 | 20 | 10 | 4 | 13 | 13 | 8 | 0 | 8 |
| B | 6 | 8 | 9 | 5 | 13 | 22 | 20 | 11 | 12 | 15 |
| C | 5 | 8 | 1 | 11 | 22 | 26 | 33 | 20 | 23 | 14 |
| D | 11 | 17 | 13 | 14 | 21 | 26 | 22 | 12 | 12 | 4 |
| E | 12 | 15 | 19 | 16 | 22 | 18 | 15 | 11 | 10 | 10 |
| F | 7 | 10 | 12 | 10 | 15 | 10 | 16 | 7 | 15 | 15 |
| G | 0 | 9 | 8 | 12 | 16 | 18 | 32 | 23 | 29 | 19 |
| H | 9 | 17 | 11 | 14 | 14 | 15 | 25 | 23 | 19 | 14 |
| I | 10 | 12 | 6 | 10 | 11 | 22 | 31 | 19 | 11 | 11 |
| J | 13 | 15 | 10 | 8 | 16 | 30 | 24 | 11 | 10 | 7 |

**P2**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 5 | 4 | 12 | 4 | 14 | 20 | 8 | 1 | 8 |
| B | 8 | 8 | 12 | 29 | 15 | 17 | 13 | 2 | 6 | 14 |
| C | 15 | 9 | 8 | 15 | 11 | 13 | 12 | 6 | 6 | 11 |
| D | 10 | 6 | 7 | 12 | 10 | 10 | 8 | 2 | 6 | 12 |
| E | 8 | 4 | 3 | 8 | 7 | 8 | 7 | 6 | 5 | 7 |
| F | 6 | 0 | 5 | 8 | 8 | 7 | 6 | 10 | 8 | 3 |
| G | 11 | 7 | 7 | 8 | 9 | 8 | 1 | 7 | 12 | 8 |
| H | 8 | 10 | 8 | 6 | 8 | 14 | 6 | 5 | 9 | 8 |
| I | 2 | 7 | 9 | 4 | 1 | 6 | 2 | 6 | 10 | 8 |
| J | 4 | 6 | 5 | 0 | 2 | 6 | 5 | 8 | 8 | 6 |

**P3**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| C | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 1 | 0 | 0 |
| D | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 0 | 0 | 0 |
| E | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| F | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| G | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 2 | 1 |
| H | 1 | 0 | 1 | 2 | 1 | 0 | 2 | 2 | 2 | 0 |
| I | 2 | 2 | 2 | 1 | 1 | 1 | 3 | 4 | 2 | 1 |
| J | 2 | 2 | 1 | 0 | 3 | 2 | 6 | 8 | 4 | 1 |

Figure 1. Heatmap of observed and estimated juvenile distribution of *H. diversicolor* in 3 independent breeding tanks (P1, 2, and 3). The blue square represents the water inlet into the tank.

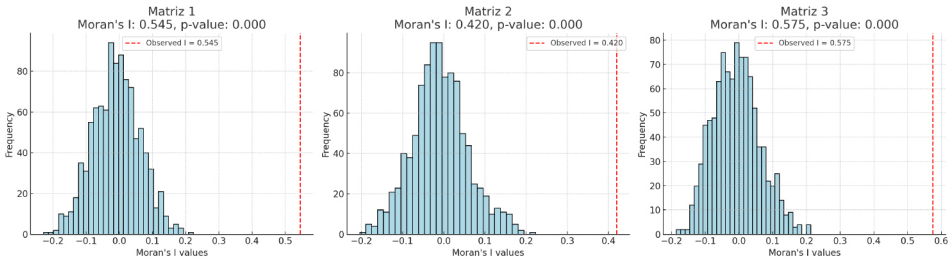


Figure 2. Results and graphical representation of Moran's index of spatial autocorrelation and associated p-value for each of the 3 data matrices of juveniles recorded in the 3 broodstock tanks.

The results showed Moran's I indices of 0.545, 0.420, and 0.575 for tanks P1, P2, and P3, respectively, with p-values of 0.000 in all cases (figure 2). Likewise, the coefficients of variation for the matrices were 49%, 56%, and 146% for P1, P2, and P3, respectively. Both results reflect the presence of strong positive spatial autocorrelations, with a tendency for clustering of cells with similar values, indicating a non-random pattern. Additionally, coefficients of variation close to or above 50% reflect high spatial heterogeneity. These results highlight the need to establish appropriate sampling techniques that adapt to the heterogeneous distribution of juveniles in the breeding tanks.

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# SELECTION OF RAW MATERIALS DERIVED FROM AGRO-INDUSTRIES FOR THE FORMULATION OF DIETS FOR *Hediste diversicolor*

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**Summary**
In this work, different wastes from agro-industries are evaluated from the point of view of growth performance and the resulting LC-PUFA composition in *Hediste diversicolor* as a preliminary step to the formulation of specific diets. Brewer spent grain, rosé wine lees and biofloc wastes were *a priori* selected on the basis of the growth performance and PUFA profile provided.

**Introduction**
The increasing interest in cultivation of the polychaete *Hediste divesicolor* is largely due to its ability to synthesise long-chain polyunsaturated fatty acids (LC-PUFA), particularly ARA and EPA, from lipid-poor substrates (Villena et al., 2025), giving it potential use as an alternative feed ingredient for fish and crustaceans. In order to achieve a production of *H. diversicolor* with an optimal nutritional composition, it is necessary to have a low-cost, high-performance feed. In the context of a circular economy, using low-lipid waste from the agri-food industry to produce a high nutritional quality product offers high levels of sustainability and business opportunity. In this work, several wastes from different agro-industries are evaluated from the point of view of growth performance and the resulting LC-PUFA composition in *Hediste diversicolor* as a preliminary step to the formulation of specific diets.

**Materials and Methods**
Eight agro-industry wastes were used to feed *H. diversicolor*, coming from brewing industry (brewing spent grain, BSW), wine industry (wine grape skins (WGSk) and stems (WGSt); rosé (ROWL) and red (REWL) wine lees); cider industry (cider apple skins, CAS) and aquaculture (biofloc wastes (BFW) and fish farming wastes (FFM)). The wastes were dried at low temperature (45 °C), grinded, and vacuum-packed for storage prior to supply. *H. diversicolor* juveniles taken form the stock of the Polychaete Aquaculture Unit (PAU) belonging to COST-IEO/CSIC with a mean body weight (BW) of 40.9 mg were placed in *ad hoc* experimental units (Aguado-Giménez et al., 2023) at a density of 1500 individuals $m^{-2}$ within a RAS system. Temperature was set to 18 °C, salinity was 35 ‰ and photoperiod was 14L:10D. Eight groups in triplicate were each fed one of the abovementioned feeds. Feed was provided *ad libitum* every two days for 3 months. At the end of the trial all specimens were (wet) weighed and specific growth rate ($SGR= (\ln BW_f - \ln BW_i)*100/ T_f-T_i$) was calculated. Protein, lipid and the fatty acid profile of all diets and of the resulting worms fed with such diets were analysed.

**Results and Discussion**
Figures 1 and 2 show the protein and lipid content in the raw materials tested. Figure 3 shows the mean SGR values for each diet. WGSk, WGSt, and CAS provided very low SGR results, and the other wastes gave similar SGR values between 2.5-3 % $day^{-1}$.

Figures 4A-C show the % of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of the diets and the resulting worms fed such diets, and Figure 5 shows the score plot of a Principal Components Analysis run with the fatty acid profiles of the diets and worms.

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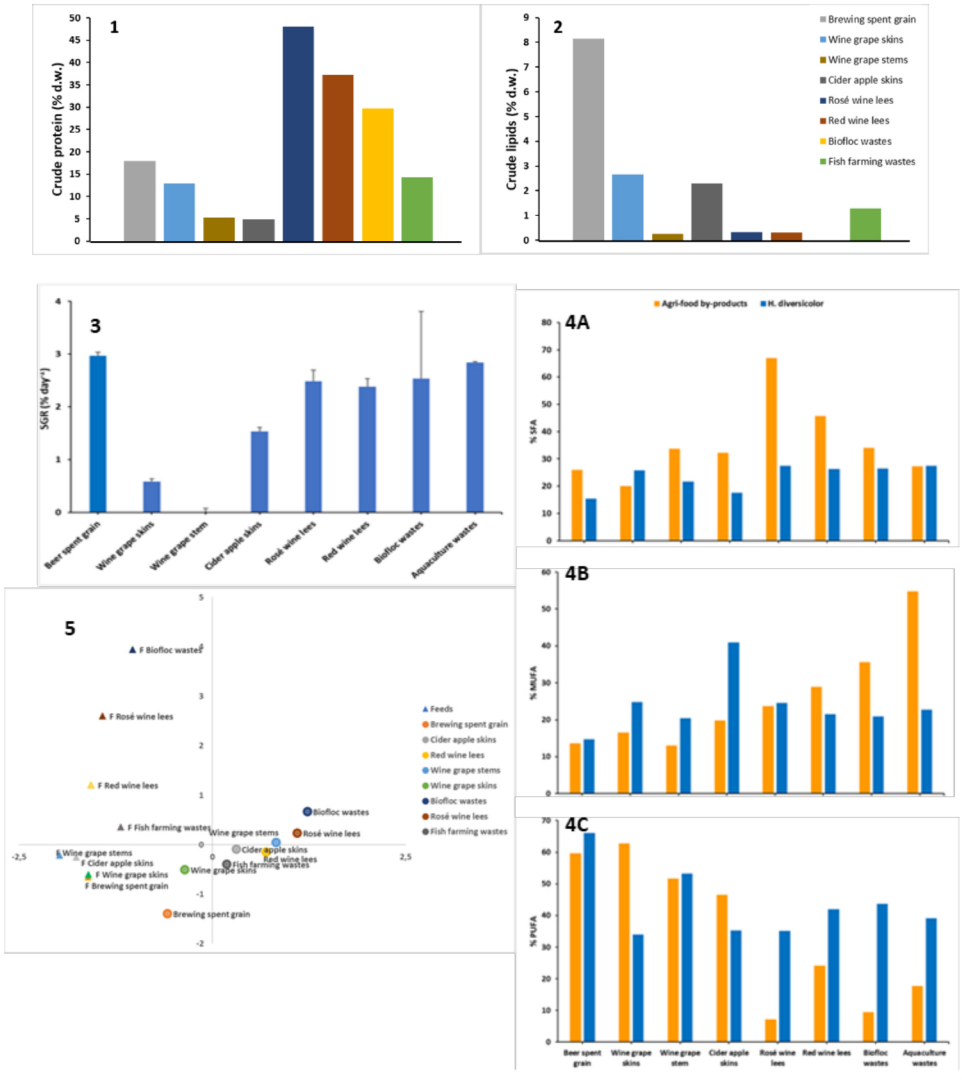


*H. diversicolor* grew best with high-protein raw materials (BSG, ROWL, REWL, BFW and FFM). In addition, these worms transformed low-lipid raw materials with a high SFA and MUFA profile (ROWL, REWL and BFW) into PUFA-rich biomass. Considering the performance provided by the evaluated raw materials and their accessibility, we selected BSG, ROWL and BFW for feed formulation with different proportions of these raw materials (and others usually used in aquaculture feed formulation to enhance protein content) that are currently being tested in terms of growth and resulting lipid and free-AA profiles.

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**Acknowledgement**

This study is part of the POLYPUFA project (PID2022-136234OB-C22) funded by the Spanish Ministry of Science and Innovation and Universities (MCIN/ AEI/10.13039/501100011033). Authors also wish to thank to the following collaborating Spanish agri-food industries: Rocker Beer Brewery (Cantabria), Bodegas Ramón Bilbao (La Rioja), and Escalante Pomological Association (Cantabria).

# MICROBIOME ASSESSMENT OF *Hediste diversicolor* FED AQUACULTURE SLUDGE: IMPLICATIONS FOR ITS POTENTIAL USE AS AN INGREDIENT IN AQUACULTURE

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**Summary:**
The microbiota of *Hediste diversicolor* was studied under three diets: formulated fish feed (FF), fresh aquaculture sludge (FAS), and freeze-dried aquaculture sludge (FDAS). Growth was highest with FF. Microbial communities differed by diet: FAS and FDAS-fed worms were dominated by *Vibrio*, while in FF worms prevailed *Fusibacter* and *Sulfurovum*. Potential pathogens like *Vibrio harveyi* and *Photobacterium damselae* were found at low levels, mostly in both sludge-fed groups.

**Introduction:**
Integrated Multitrophic Aquaculture (IMTA) offers a sustainable model using polychaetes like *Hediste diversicolor* to convert organic waste into feed (Wang *et al*., 2019). Polychaete meals can replace fishmeal without harming fish growth, but EU rules limit use of potentially hazardous materials like fish feces. Assessing microbial risks is crucial, and 16S rRNA sequencing helps understand how diet affects *H. diversicolor* microbiota, in order to support its safe use in aquaculture (Austin and Austin., 2016).

**Material and Methods:**
This 46-day experiment involved 360 *Hediste diversicolor* juveniles reared in cylindrical experimental units within recirculating aquaculture systems under controlled conditions (18 °C, 35 PSU, 16L:8D). Three diets were tested: commercial fish feed (FF), and fish wastes (mix of faeces and uneaten feed) in two formats: fresh aquaculture sludge (FAS), and freeze-dried aquaculture sludge (FDAS), each with six replicates. Worm growth was measured every 15 days using specific growth rate (SGR). For microbiota analysis, DNA was extracted from whole worms, culturable bacteria grown on selective media, the diets, and seawater. The V3–V4 region of the 16S rRNA gene was sequenced via Illumina MiSeq and processed with QIIME2. Diversity indices (Shannon, OTU richness) and beta diversity (Weighted UniFrac) were calculated, and dominant taxa identified at phylum and genus levels. Potential pathogens were screened by BLASTn against SILVA references (≥ 98.7 % identity). Growth data were analyzed using repeated measures ANOVA in R.

**Results:**
The growth analysis of *H. diversicolor* revealed marked differences depending on the diet provided (Figure 1). Worms fed with FF showed enhanced growth compared to those fed with FAS and FDAS, suggesting a better nutritional suitability of natural substrates. Regarding the associated microbiota, principal coordinates analysis (PCoA, Figure 2) showed a clear separation between treatments. Worms fed with FF developed a microbial community that diverged more strongly from the initial state, while those fed with sludge maintained bacterial profiles closer to the original microbiota. Relative abundance bar graphs (Figures 3A-B) indicated that the genus *Vibrio* largely dominated the initial microbiota (~97%) and remained predominant in worms fed with FAS and FDAS. However, its presence decreased significantly in the FF-fed group (24%), allowing other genera such as *Fusibacter*, *Sulfurovum*, and *Desulforhopalus* to proliferate. In cultured samples, *Vibrio* was also the most abundant genus (47–78%), although it shared dominance with *Acinetobacter*, *Psychrobacter*, or *Pseudomonas*, depending on the diet. Finally, feed samples (Figure 4A-B) revealed that sludges contained bacterial genera also found in the worms, including *Vibrio*, *Psychrobacter*, and *Fusibacter*, as well as traces of plant-origin DNA. In culture media and seawater, *Vibrio* was also predominant, reinforcing its role as a central component of both the environmental and host-associated microbiota.

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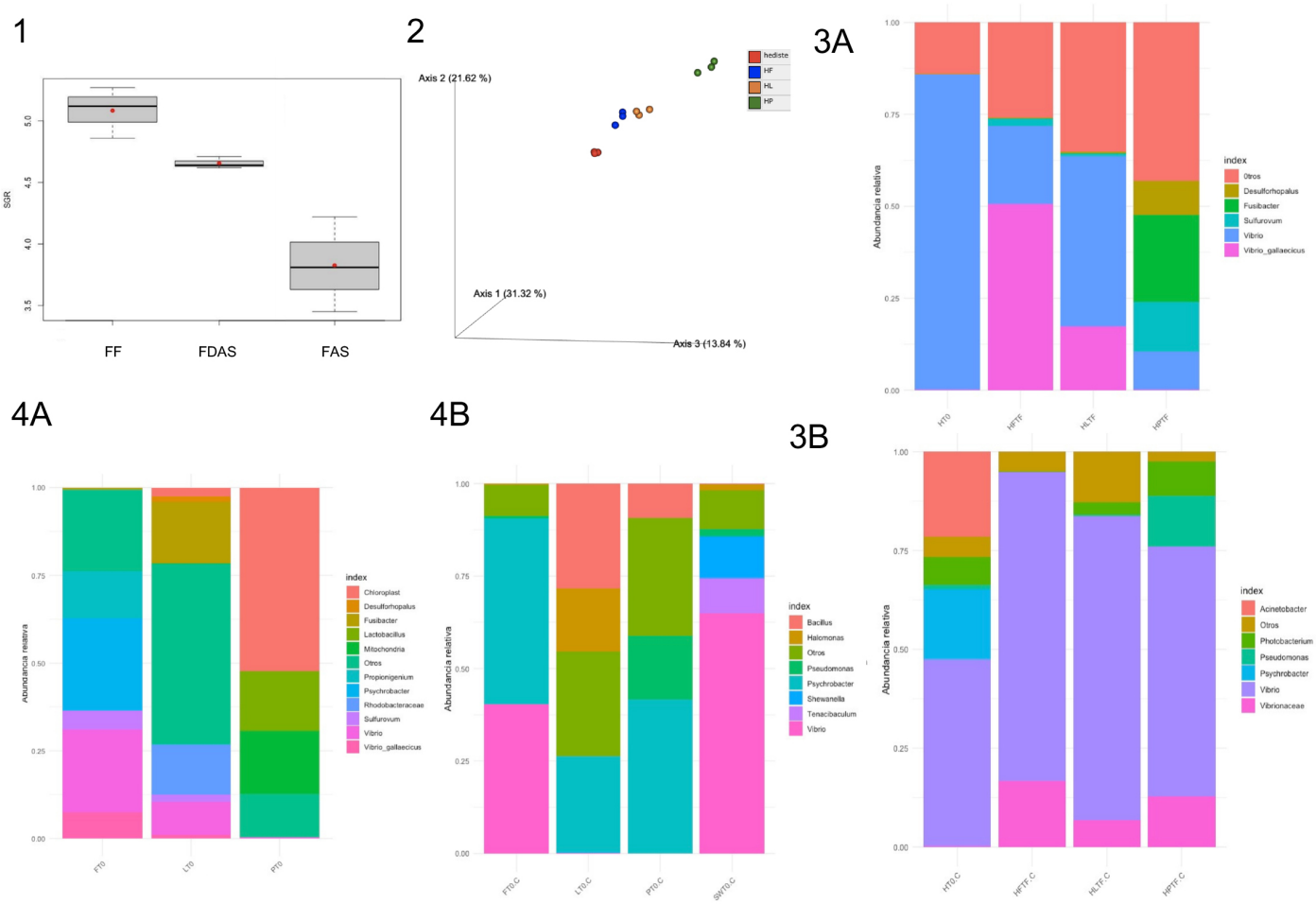


*H. diversicolor* microbiota is dominated by Proteobacteria, mainly *Vibrio*. Diet impacts its composition: sludge-fed worms retain the original community, while pellet feed increases anaerobes like *Fusibacter*. No major human pathogens were found, but potential fish pathogens were present in worms and sludge, indicating possible transmission risks.

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**Acknowledgement**

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# INFLUENCE OF DENSITY ON GROWTH, FEEDING RATE AND EFFICIENCY IN *Hediste diversicolor*

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**Summary**
This study reveals the strong influence of density (D) on mortality (M), growth (SGR), feeding rate (SFR) and efficiency (FE) in *Hediste diversicolor*, as function of bdy weight (BW) and temperature (T). As D and BW increase M also increases but SGR decreases, but markedly from 8000 ind. $m^{-2}$. SFR and FE were also negatively correlated with D. SFR increased with T but FE decreased. Splitting during the fattening process leads to greater biomass production in a shorter time.

**Introduction**
Density is a determining factor in the structuring of benthic communities, particularly polychaetes. Under aquaculture conditions it is also of great importance for welfare, production performance and stock management. In this study we assess the influence of density on growth performance, feeding rate and efficiency in *Hediste diversicolor* of a wide range of body weight and temperature.

**Materials and Methods**
**Trial 1: Effect of individual size and density on growth**. Specimens of six different weight classes (10–25, 25–50, 50–100, 100–150, 150–250 and 250–500 mg fresh body weight (BW)) were placed in separate experimental units (EU) at eight different densities (D: 500, 1000, 2000, 4000, 8000, 12 000, 14 000 and 16 000 ind $m^{-2}$). All weight classes were represented in triplicate at the different densities in all trials. Worms were fed *ad libitum* with sole weaning pellets. After a period of 15 days, mortality (M: %), growth (SGR: % $d^{-1}$) and biomass production (BP: g $m^{-2}$) were calculated. Two-way ANOVA was used to check for the interaction effect of D x BW on M. Multiple regression analysis (MRA) is used to relate SGR to D and BW in order to obtain a predictive equation. Simulations are used to show production performance under different scenarios.

**Trial 2: Effect of individual size, water temperature and density on feeding rate (SFR) and feeding efficiency (FE)**. Specimens of six different weight classes (10–50, 50–100, 100–150, 150–200, 200–400 and >400 mg fresh BW) were placed in separate EUs at four different densities (D: 355, 1059, 1765 and 2471 ind $m^{-2}$) at three different temperatures (T: 14, 18 and 22°C). All weight classes were represented in triplicate in all trials at the different densities and temperatures. Before beginning the trials, the specimens in each EU were deprived of food for three days. Afterwards, they were fed daily at a high rate to ensure food was always in excess. The number of feed pellets (mean pellet weight previously measured) provided was pre-counted. At the start of each day, the EU surfaces were photographed under blue light, and leftover pellets were counted with ImageJ2 software. The number of pellets eaten the prior day was replaced to maintain continuous access to feed. This routine continued for four days, after which the animals were weighed and returned to their EUs. The entire process—three days of fasting and four days of feeding—was then repeated once. SFR and FE was calculated according to feed intake. The three-day fasting period between feeding tests ensured that all specimens would begin the next feeding trial with similar hunger levels, preventing repeated measure bias. Multiple regression analysis is used to relate SFR and FE data to D, T and BW

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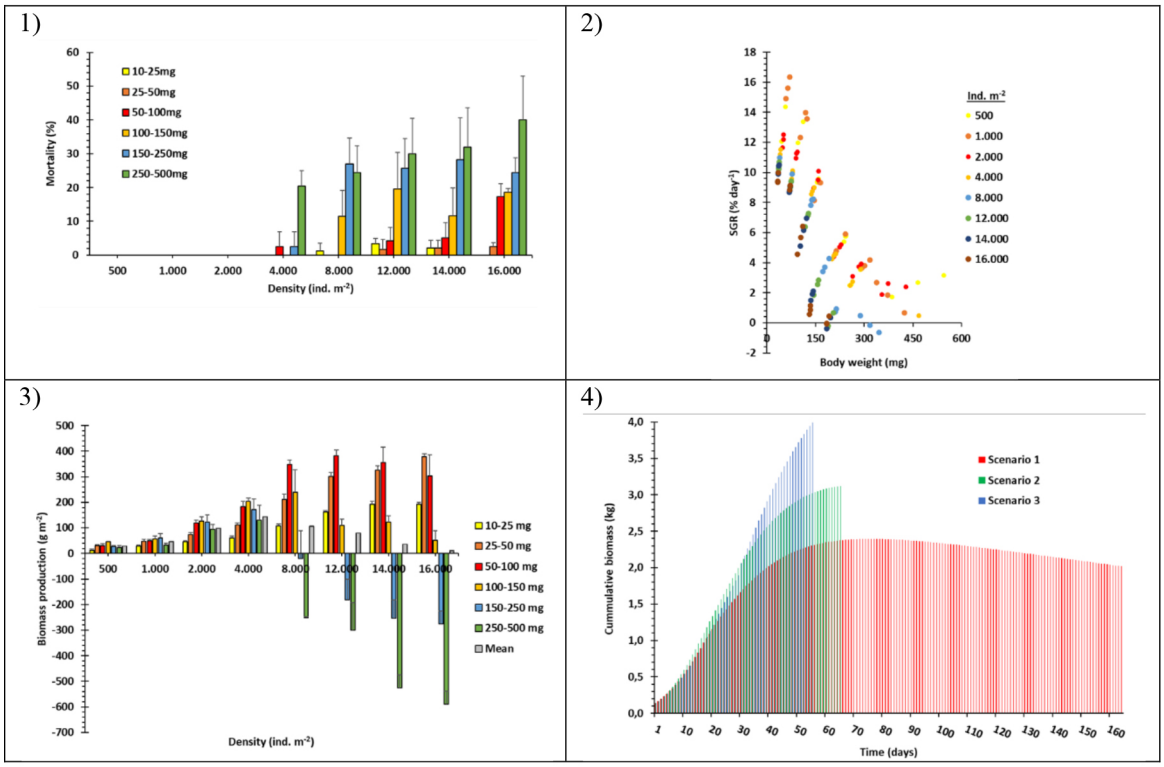


**Results and Discussion**

D x BW interaction on M was significant ($P < 0.001$), so that the higher the D and BW, the higher the M. From a D of 8000 ind. $m^{-2}$ and a BW greater than 150 mg, the observed M is nearly 40%. (Figure 1). MRA revealed a significant and negative interaction of D x BW on SGR (SGR = 33.89 - 5.08·Ln(BW) - 8.52·10-5·D·Ln(BW); $R^2$ adj.: 0.79; $P < 0.0001$). As BW and D are low, SGR tends to be higher, and the opposite is also true. Once D exceeds 8,000 ind. $m^{-2}$ and BW is above 180 mg, SGR turns negative: as density increases further, growth becomes even more negative (Figure 2). BP increases as density and weight increase, but above 8000 ind. $m^{-2}$, low growth and mortality do not compensate the BP for BW > 150 mg (Figure 3). Simulations for BP were performed under different scenarios: SC1) initial D of 8000 ind $m^{-2}$ without any population split; SC2) initial D of 4000 ind $m^{-2}$ without any population split; SC3) initial seeding at a D of 8000 ind $m^{-2}$, with a first splitting at a D of 4000 ind $m^{-2}$ when worms reach a mean BW of 150 mg and a second splitting at a D of 2000 ind $m^{-2}$ when reaching a mean BW of 250 mg. Simulations revealed that splitting allows to obtain larger BP in a shorter time (Figure 4).

Similarly, MRA revealed that as BW and D decreased, SFR and FE increased: Ln(SFR) = 2.77 – 0.31·Ln(Bw) + 0.91·Ln(T)-0.34·Ln (D) + 5.4· 10-5·D·Ln(Bw); ($R^2$adj = 0.54; $P < 0.0001$ Ln(FE) = 10.02 – 5.1·10-4·Bw -0.02·T – 0.82·Ln(D) - 3.5·10-5·D·Ln(Bw); $R^2$adj = 0.81; $P < 0.0001$. With regard to temperature, as it increases, the SFR also increases but the FE decreases.

Density significantly affects *Hediste diversicolor* development, with small individuals (<150 mg) exhibiting rapid growth even at high densities, while larger specimens experience reduced growth and survival at increased densities. Adjusting densities through splitting as the worms grow shortens production cycles and minimizes mortality but increases space requirements. Feeding rate and efficiency are also strongly impacted by density, temperature, and body weight.

**Acknowledgement**

Trial 1 is part of ThinkInAzul project, financed by by the Spanish Ministry of Science and Innovation with European Union NextGenerationEU funds, and by the Government of Cantabria (Spain). Trial 2 is part of the POLYPUFA project (PID2022-136234OB-C22) funded by the Spanish Ministry of Science and Innovation and Universities (MCIN/AEI/10.13039/501100011033).

# OFFSHORE LOW-TROPHIC AQUACULTURE MULTI-USE REALISATION (OLAMUR): PRODUCTION POTENTIAL AND ECOSYSTEM SERVICES OF LOW TROPHIC AQUACULTURE AT MULTI-USE AREAS IN THE BALTIC AND NORTH SEAS

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The recent expansion of offshore wind power has emphasized conflicts among different stakeholders. This need for more efficient use of marine area has highlighted the potential for multi-use, i.e. the use of area for more than one purpose. Aquaculture of low-trophic species (LT) (non-fed aquaculture) appears as an attractive way to increase sustainable biomass production. Additionally, LT species such as bivalves or seaweeds provided diverse ecosystem services such as the removal of nutrients or water clarity. Thereby, LTA can improve water quality and deliver ecosystem services, while contributing to the circular bioeconomy. As such activities may be combined in the same area, there has been considerable interest in developing multi-use systems for both industries, contributing to a range of UN Sustainable Development Goals (SDGs).

In the OLAMUR project, we are developing initiatives for multi-use involving low-trophic aquaculture (LTA) in the Baltic and North Seas. with offshore wind, combined with cultivation of mussels and seaweeds. The Baltic case is situated at Kriegers Flak, in the Danish sector, and the North Sea case is north of the island of Helgoland in the German sector. At both sites, large scale offshore wind farms have been developed and are now in the production phase. The Baltic case started seaweed cultivation in 2023, and mussel cultivation in 2024, and the German case starts in 2024. A third multi-use case study, in Estonian waters, combines cultivation of seaweeds and mussels with rainbow trout farming.

Among other specific objectives, OLAMUR aims to assess the suitability of the production of LT species in the multi-purpose use locations assessing the production potential and the efficiency of co-use scaled farming scenarios (OWF and IMTA) represented by the 3 established pilots. OLAMUR uses state-of-the-art ecological modelling approaches coupled with Dynamic Energy Budget Individual Based population models (DEB-IBM) for mussels, seaweed and fish. These models are validated through the data gathered at the pilots and are used to assess the farm-scale spatial production patterns and potentials for providing ecosystem services (uptake of nutrients). The models link local environmental conditions with the physiological performance of the crop to allow estimations of spatial and temporal variability of the crop status and are used to compare the different pilots, different farming scenarios and the development of tools for site selection.

OLAMUR runs a databased service system to assist policymakers in making knowledge based decisions, and an extensive Data Management Plan ensures transparent and standardized data capture from all parts of the project.

# GENOMIC ADAPTATIONS OF *Litopenaeus vannamei* IN AN INDOOR BIOFLOC AQUACULTURE SYSTEM

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**Introduction**
Biofloc technology (BFT) enhances water quality, nutrient recycling, and disease resistance in shrimp aquaculture through microbial activity. This study investigates the gene expression responses of Pacific whiteleg shrimp, *Litopenaeus vannamei*, to varying biofloc conditions in an indoor system. This research focuses on molecular adaptations essential for shrimp health and sustainable production.

**Materials and Methods**
Tissue samples from *L. vannamei* were collected from three treatments (GRG 1: 200 shrimp/$m^3$, C/N ratio 10, 14 ml/L probiotic; GRG 2; Control), each with three replicates. RNA was extracted following the methods described in Masoodi et al. (2020), treated with DNase, and quality-checked (260/280 ratio: 1.9–2.1). mRNA was purified, fragmented, and converted to cDNA. Libraries were sequenced on an Illumina platform, with raw reads filtered using FastQC, MultiQC, and Cutadapt. Clean reads (>97%) were aligned using HISAT2, and differentially expressed genes (DEGs) were identified via DESeq2 ($|\log_2(\text{FoldChange})| \geq 1$, padj $\leq 0.05$). Functional enrichment used ClusterProfiler with KEGG annotation to indicate the pathways of biological significance in each set of DEGs.

**Results**
Gene expression analysis identified substantial DEGs: 1245 in GRG 1 vs. Control; 1145 in GRG 2 vs. Control; and 681 in GRG 1 vs. GRG 2 (Figure 1). Figure 2 also indicated distinct DEGs in the biofloc treatments relative to control. GRG 1 vs. Control showed upregulated proteasome (Rpn5, β4), autophagy (LC3 up 5-fold), and phagosome (Tubulin α up 10-fold) pathways. GRG 2 vs. Control showed increased level of aminoacyl-tRNA biosynthesis (alanyl-tRNA synthetase) and endoplasmic reticulum protein processing (SEC23/24 up 5-fold).

**Discussion**
GRG 1 biofloc conditions significantly enhanced shrimp physiology. The proteasome pathway showed upregulated subunits (Rpn5, β4) indicating an improvement in protein degradation and cellular homeostasis as earlier reported by Said *et al*., (2022). Autophagy pathways showed upregulation (4–5-fold) of LC3 and TSC1 to represent enhanced stress resilience as shown in earlier studies by Broadbent et al., 2023). Phagosome pathways showed upregulation (5–10-fold) for Tubulin α and integrin suggesting a boost in immune responses empirically verified by Mao *et al*., (2020). Upregulation of Alanyl-tRNA synthetase in Aminoacyl-tRNA biosynthesis pathway of GRG 2 supported protein synthesis. Endoplasmic reticulum protein processing enhanced protein trafficking with the upregulation of SEC23/24 and Hsp70 earlier confirmed by Belenichev *et al*., (2023).

**Conclusion**
The significant upregulation favouring GRG 1 biofloc treatment for proteasome, autophagy, and phagosome pathways in *L. vannamei* confirmed improved stress management and immunity. GRG 2 on the other hand supports protein synthesis and trafficking. These genomic insights confirm the role of BFT in improving shrimp health and advancing sustainable aquaculture.

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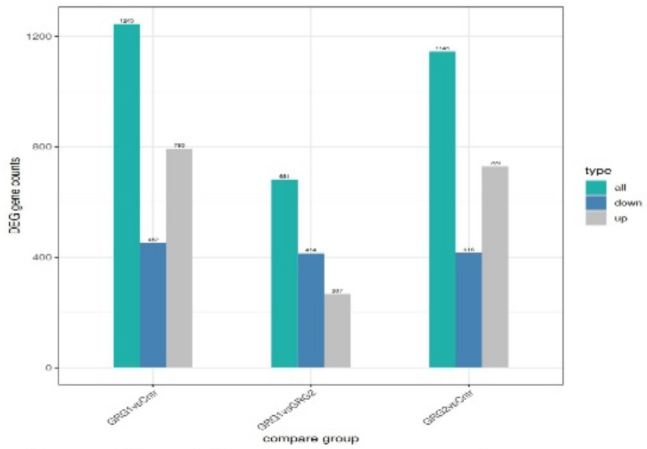


Table 1: Differentially expressed genes (DEG) count of the treatments

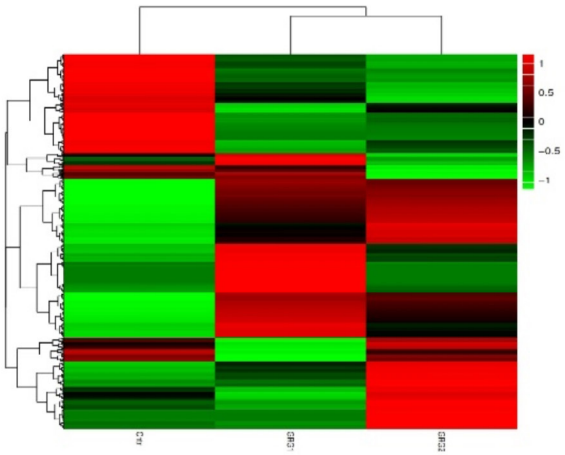


Figure 2: Cluster diagram showing the differentially expressed genes in each treatment

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# INVASION OF THE BLUE CRAB (*Callinectes sapidus*) IN ITALIAN WATERS: A REVIEW OF CURRENT KNOWLEDGE, IMPACTS, AND MANAGEMENT GAPS

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The blue crab (*Callinectes sapidus*), a species native to the western Atlantic, has become an aggressive marine invader in the Mediterranean, with Italy experiencing particularly severe ecological and economic consequences. This review synthesizes recent literature (2010–2024), gray reports, and unpublished data on the distribution, biology, ecological impact, and management responses to the species' spread along Italian coasts, especially in the northern Adriatic, central Tyrrhenian, and Sicilian lagoons. Key studies report rapid population increases, with reproductive females and juveniles found throughout most surveyed lagoons. Reviewed methodologies include baited trap surveys, remote sensing for habitat mapping, stable isotope analysis, and socioeconomic assessments via fisher interviews. Impacts identified include predation on native bivalves (e.g., *Ruditapes philippinarum*), competition with native crustaceans, and disruption of aquaculture and artisanal fisheries. While several regional mitigation strategies such as population control through targeted fishing have been trialed, a coordinated national response is lacking. Notably, there is minimal integration of ecological data into policy, limited market development for blue crab as a fishery resource, and inadequate long-term monitoring programs. This review highlights the urgent need for multidisciplinary research and management approaches that address both ecological and socioeconomic dimensions of the invasion. Critical gaps remain in understanding larval dispersal mechanisms, overwintering survival, and adaptive ecosystem responses, which are essential for predicting future expansion and informing policy.

# OCEAN FISH FARMING – ENSURE SAFE AND SUSTAINABLE OPERATIONS

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## INTRODUCTION

Oceans space offers a vast opportunity to meet marine protein demand with sustainable, safe and efficient fish farming, but how do we maintain safety of facility, personnel on board and prevent fish escape together with maintaining fish welfare and reducing emissions to marine environment. Adding safety to fish farming operations is mainly ensured by providing uniformity, transparency, and predictability and thereby securing the total safety performance. This paper reflects and address overall risk perspectives combined with applicable taxonomy of essential items crucial for maintaining safe aquaculture operations in ocean space.

## MAIN APPROACH

The main areas of concern when it comes to ensuring safe and reliable fish farming units may be categories into: Integrity of facility, personnel safety, fish welfare, maintaining ocean health and prevention of fish escape. An integrated risk methodology based on these four items results in a cost-efficient approach to reduce operational risk and establish sufficient safety level.

### Integrity of facility

Integrity of facility includes structural strength, stability, mooring, technical arrangement, and solutions on board together with reliability of essential equipment installed. Integrity of vessel or installation itself is directly relevant to existing scheme for qualification or classification. Experience from more than 50 years of design and operation of complex steel structures in demanding marine environment are compiled in the classification rules. This maritime execution model has been applied in the six first fish farming installations put into operation in Europe.

### Personnel safety

Personnel safety is mainly addressing arrangement for emergency escape and fire safety. Maintaining Health, Safety and Environment for personnel working on board a fish farming installation is demanding since challenging task are performed daily in hash, marine environment. Special considerations need to be taken due to offshore/maritime operational mode. This included lifesaving appliances, launching equipment and similar as well as fire detection and -extinguishing. It is common to apply well know maritime IMO-codes as acceptance criteria for personnel safety. These codes also describe certification process and how to prototype test to ensure reliable safety equipment on board. SOLAS is a good example followed by local flag- or shelf states interpretation of requirements embedded in this maritime code.

### Fish welfare

Fish welfare and requirements related to this varies depending on local authorities. It is essential to verify the reliability of technology utilized to monitor environment of the fish. Instrumentation indicating oxygen level, temperature, salinity, turbidity is subject for special attention. Maximum acceptable level of biomass is also a crucial parameter that needs to be monitored.

In general capability and reliability of the equipment and systems on the installation that affects welfare, health and physical condition of finfish need to be addressed systematically. As a result, items for fish welfare on board such as cleaning, exposure to chemicals, medication, crowding, fish transfer and monitoring of health condition are of the essence.

### Ocean health

A significant risk in ocean aquaculture is substandard sustainability due to emissions from fish farming installation. It is considered essential to monitor and limit pollution from e.g. fish waste, feed surplus, chemical substances from net cleaning and emissions from medical treatment of feed stock. This to ensure that environmental impact of fish farming operation does not exceed the sustainability limits of marine environment. Sustainable aqua operation is mainly achieved through monitoring performance and ensuring that biodiversity targets for marine environment are reached.

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**Fish escape prevention**
Fish control or prevention of escape is the main function of a fish farming unit. Structural integrity of net system and ropes together with capability of fish transfer systems are crucial items in fish control. Flexible net systems utilized in rigid high volume steel fish farming installation has proven to be exposed to fatigue and need to be attended to in particular. Wear and tear of net due to cleaning and handling is also a concern. Several of reported incidents related to fish escape happens while handling of fish – for example crowding due to de-licing or transfer. Equipment contributing to these operations needs to be specially attended to.

In addition to arrangement representing physical barriers preventing the fish to escape, all other systems and components that may cause fish escape if they fail or malfunction in any way need to be certified.

The four different items listed above, asset integrity, personnel safety, fish welfare and fish control, are considered equally important for safe and sustainable fish farming offshore. These elements are also closely interconnected where integrity of one may support several others.

**CONCLUSION**
There is a significant potential to utilize competence from traditional offshore and maritime industry to help operators of exposed fish farming units to identify operational risks by applying technical rules and requirements from traditional maritime execution model.

Combining the well-known certification concept from maritime industry with balanced aquaculture-based requirements for fish welfare and biodiversity provides a robust and cost-efficient solutions to reducing risk in operation of ocean fish farming installations.

# GLOBAL OCEAN HEALTH STANDARD - SUPPORTING SUSTAINABILITY IN AQUACULTURE

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## INTRODUCTION

Marine aquaculture has grown into a vital industry, providing an alternative source of seafood while supporting the global animal-source protein supply worldwide. However, with this rapid expansion comes a pressing need for consistent regulations, improved aquaculture facilities, and sustainable operations to ensure long-term viability.

A global ocean health standard focusing on controlling and reducing emission from aquaculture activity to marine environment is one of many initiates to improve sustainability in global aqua-industry.

## MAIN APPROACH

An ocean health standard is established and serves as a key framework for certifying and improving sustainability, and establishes best practices for designing, constructing, and maintaining aquaculture facilities to ensure environmentally responsible operations. It outlines comprehensive measures to ensure the health and welfare of farmed fish stocks while limiting the overall environmental impact from aquaculture operations. Some of the key drivers to implement requirements in this standard include:

- Sustainability & environmental responsibility: Strategies to mitigate the impact of fish farming on surrounding ecosystems, including waste management and water quality control.
- Operational efficiency & cost optimization: Guidelines for optimizing farming operations to maximize productivity while maintaining sustainable practices.
- Market competitiveness & business growth: Certification with focus on monitoring and limiting discharge from aquaculture operations, helping fish farmers differentiate themselves in the rapidly emerging and competitive aquaculture markets, through improved sustainability, operational efficiency, transparency, and operative predictability.

By adopting to these requirements, fish farming companies may potentially improve consumer trust by increased sustainability through enhanced operational performance and reduced environmental impact. This is mainly achieved through limiting the discharge to the marine environment from aquaculture operations.

As global seafood demand rises, sustainability has become an essential aspect in current aquaculture. Poorly managed fish farms can cause significant negative environmental impacts related to e.g. spread of infectious agents, fish escape as well as nutrient- and chemical discharge, leading to water pollution, destruction of marine ecosystems and biodiversity loss. Therefore, we see that fish farming industry is rapidly developing innovative solutions to face these challenges.

This ocean health standard addresses marine environmental concerns by requiring a site-specific biosecurity plan for each production cycle, with special attention to local conditions. Additionally, it mandates a baseline survey of the installation- and transition zones before aquaculture facility is installed, and/or before substantial expansion. This provides a benchmark to measure changes over time, allowing for the detection of trends and shifts in environmental conditions. Furthermore, this set of requirements also encourages improved waste management through the implementation of a waste plan for waste storage and treatment for sewage and solid waste together with sludge collection. These measures illustrate some examples of how the standard ensures that fish farms contribute to cleaner water and healthier aquatic habitats.

## CONCLUSION

The aquaculture industry faces increasing demands for food security, environmental responsibility, and technological advancement. As we move forward, continued innovation and adherence to best practices will be essential to maximize the potential of fish farming and ensure its role as a sustainable source of seafood. Compliance with consistent requirements such as the standard DNV-ST-0678 “Environmental impact of marine aquaculture installations” will be necessary for the aquaculture sector to achieve long-term success. Ultimately, this standard serves as a roadmap for responsible, high-performance aquaculture that can meet the challenges of the next decade and beyond.

# STRESS-INDUCED DISRUPTION OF CIRCADIAN SEROTONIN SYNTHESIS IN THE GUT OF RAINBOW TROUT (*Oncorhynchus mykiss*): EXPLORING L-TRYPTOPHAN AS A PREVENTIVE STRATEGY

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**Introduction**

Welfare is critical in aquaculture, especially when considering that farmed fish can experience stress. Stress negatively affects a wide range of physiological and behavioural functions, including disruption of rhythmic processes that are largely dependent on the circadian system. The gastrointestinal tract (GIT) plays a critical role in nutrient absorption and energy homeostasis, and its regulation involves multiple neuroendocrine processes. Several monoamines display an important role in gut, with special relevance of serotonin (5HT). In fish, this monoamine participates in the modulation of feeding behaviour, but also in the physiological stress response. In addition, 5-HT specifically regulates several functions within the GIT, such as motility, secretion, and daily rhythms through specific receptors. Its synthesis takes place from the essential amino acid L-tryptophan (L-Trp), whose role in contributing to animal welfare is expected. Dietary supplementation with L-Trp appears to suppress aggressive behaviour and reduce circulating cortisol after acute stress in some teleost species (Höglund *et al*., 2019), limiting its synthesis and release in long-term trials (Herrera *et al*., 2019). In addition, L-Trp promotes synthesis of intestinal melatonin, which may influence the circadian regulation and the physiological response to stress (Acharyya *et al*., 2021). However, the benefits of L-Trp are not demonstrated at all, and it has been also reported to enhance plasma cortisol levels in rainbow trout (Lepage *et al*., 2002), thus negatively affecting different regulatory systems, such as the serotonergic system, which is involved in GIT immune function (Machado *et al.*, 2019). Then, there is a need to deep into the understanding of the mechanisms involving L-Trp in GIT, and to corroborate if dietary supplementation with the amino acid is effective on preventing the adverse effects of stress in farmed fish.

Therefore, this study aimed to evaluate how prolonged crowding stress affects the gastrointestinal serotonergic system in rainbow trout, and the potential benefits of dietary supplementation with L-Trp on protecting serotonergic activity from stress.

**Materials and Methods**

Immature rainbow trout (*Oncorhynchus mykiss*) were obtained from a local fish farm and kept at the University of Vigo. Fish were acclimated to a 12L:12D photoperiod, with daily feeding at ZT3 (ZT0 = lights on), and then divided into four groups regarding the stress and feeding paradigms: 1) Control (no stress) fed with control feed (0.2% L-Trp) (CC); 2) Control fed supplemented feed (1% L-Trp) (TC); 3) Stress fed with control feed (CS); 4) Stress fed with supplemented feed (TS). Supplementation of the diet with L-Trp began 7 days prior to the stress by overcrowding being applied. To stress the fish, the volume of water in the tanks was reduced to reach a high stocking density (70 Kg of fish/100 L) and remained up to 96 hours. Non-stressed trout remained in normal stocking density (10 Kg/100 L). On the day of sacrifice, groups of fish were euthanized every 4 hours throughout the following 24 h (ZT0, 4, 8, 12, 16, 20, and 0'), and individual samples of blood and foregut were collected. Plasma obtained from the blood samples was assessed for cortisol levels by EIA. The samples of foregut were used to quantify 5-HT levels by HPLC, as well as the relative mRNA abundance of tryptophan hydroxylase (*tph1*, *tph2*), the rate-limiting enzyme in serotonin, by qPCR. Significant differences between the experimental groups were determined using ANOVA and post hoc multiple comparisons test. In addition, the consistency of the daily rhythms of each parameter and their characteristics were evaluated by the COSINOR analysis.

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**Results and discussion**

In non-stressed group, a daily rhythm of cortisol levels was observed, with the acrophase (time of the peak) located at the end of the night. In fish fed with regular feed and subjected to overcrowding (CS group), the acrophase shifted and relocated in antiphase (ZT10.53) with that of control group. The amplitude of the rhythm raised up to 50 % and the mesor duplicated. In stressed trout fed with L-Trp supplemented feed (TS), the rhythm was not consistent due to decreased amplitude, but the profile paralleled that of the control group. The rhythm of cortisol in non-stressed group fed with the L-trp supplemented diet (TC) remained, with slight changes consisting of a nearly 3-hours shift of the acrophase, and a slight increase of amplitude and mesor. With respect to cortisol, non-stressed fish showed lower mean daily levels than those fish subjected to stress. In addition, the group receiving L-Trp (TC) showed slightly higher values than the control. Concerning 5-HT levels in the foregut, a significant daily rhythm was denoted in the control group (CC) with the acrophase occurring at ZT10.73. Under stress conditions (CS), the rhythm was not consistent, but the profile was similar to that of control. L-Trp was partially effective in preventing the effect of stress on the rhythm of 5-HT (TS group), although the acrophase changed. Non-stressed trout fed with supplemented feed (TC) preserved the daily rhythm of 5-HT, but the acrophase was in antiphase with respect to that of control group. Finally, with respect to *tphs* mRNA abundance, daily rhythms occurred in control group. Stress inhibited the expression of both *tph1* and *tph2*, and L-Trp supplementation was effective on preventing the effect of stress, even when slight variations on the acrophase occurred when compared to control group.

Taken together, our results suggest that L-Trp may protect the serotonergic system from tress, thus suggesting a potential use as preventive strategy in the aquaculture context, when stressful manipulations are expected.

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# DECONTAMINATION OF FOODBORNE PATHOGENS IN EDIBLE ALGAE USING COLD PLASMA

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**Introduction**

Edible algae are gaining importance in sustainable agri-food systems, with over 20 species recently authorised as novel foods in Europe[1]. However, their microbiological safety poses some concerns due to sporadic outbreaks linked to pathogenic bacteria and enteric virus contamination. This study investigates the identification of the microbial risks associated with edible algae and the implementation of cold plasma (CP) treatments, as a promising technology effective in preventing and controlling foodborne diseases.

**Material & Methods**

First, the microbiological analysis of diverse species of edible algae, either fresh and dried, was carried out for mesophilic aerobic bacteria (ISO 4833-1:2003), yeasts and moulds (ISO 21527-1:2008), *Enterobacteriaceae* (ISO 21528-2:2017), *Escherichia coli* (ISO 16649-2:2001), *Listeria* spp. (ISO 11290-2:2017), *Salmonella* spp. (ISO 6579-1:2017), *Vibrio* spp. (ISO 21872-1:2017), and *Clostridium* spp. (ISO 15213-2:2023). As well, enteric viruses were targeted according to the BAM FDA method for GI and GII human noroviruses (HuNoV), hepatitis A virus, astrovirus, human sapovirus (HuSaV), rotavirus, and mengovirus, as a process control. The efficacy of CP treatments generated by a dielectric barrier discharge (DBD) device was then evaluated for the inactivation of selected foodborne pathogens under varying operating conditions (e.g., exposure time, power, and gas composition) in algae. The efficacy of the treatment was assessed by plate count for *Listeria monocytogenes* and replication in cell culture for viruses, including murine norovirus (MNV) as cultivable surrogate.

**Results**

The microbiological analysis showed the presence of aerobic mesophilic bacteria (6.84 log CFU/g), moulds and yeasts (5.48 log CFU/g) in all fresh and dried algae samples. Potential foodborne pathogens, such as *Enterobacteriaceae*, *E. coli*, *Listeria* spp., *Salmonella* spp., and *Vibrio* spp., were detected in both fresh and dried algae, with presence varying depending on the sample. Dried algae were found to be contaminated with *Clostridium* spp (Fig. 1). Non-quantifiable HuNoV GII was detected in all fresh and dried *Ulva lactuca* samples (Fig. 1).

CP with $O_2$ resulted in a time- and power-dependent *Listeria monocytogenes* reduction in algae (Fig. 2A), reaching complete inactivation after 30 minutes at 300 W. CP with $N_2$ at 300 W resulted in the best operative conditions for MNV inactivation (>4 log, Fig. 2B), while $O_2$ completely inhibited HuNoV II replication after 20 min (4.9 log, Fig. 2C). CP at 300 W determined HuSaV complete inactivation after 20 min irrespective of the gas composition (2.5 log, Fig. 2D).

**Discussion**

This study showed bacterial and viral contamination in both fresh and dried algae, which levels are consistent with previous reports for marine products[2, 3]. The presence of *Enterobacteriaceae* and HuNoV suggests that faecal contamination occurs in both indoor and outdoor cultivation systems. Also, *Vibrio* spp. detection raises concerns as one of the zoonotic agents to be mandatorily monitored in sea products (2003/99/EC). Given such contamination, CP was hypothesized as an effective no-thermal decontamination technology for pathogen control in algae[4, 5]. Non-thermal treatments such as UVC light and gamma radiation have been shown to reduce hepatitis A virus and murine norovirus viral loads by up to 3 log in various edible seaweeds, without affecting their sensory quality[6, 7]. Cold plasma has proven effective for viral inactivation, reducing 4.22 log of MNV on raspberries after 5 minutes, and >5 log on blueberries after 1.5 minutes[8, 9]. Testing the combination of different operating conditions, our results demonstrated the complete inactivation of human bacterial and viral foodborne pathogens after 20-30 min exposure at 300 W with $O_2$. These advances serve to consolidate CP as an innovative alternative for improving the microbiological safety of novel food products such as edible algae.

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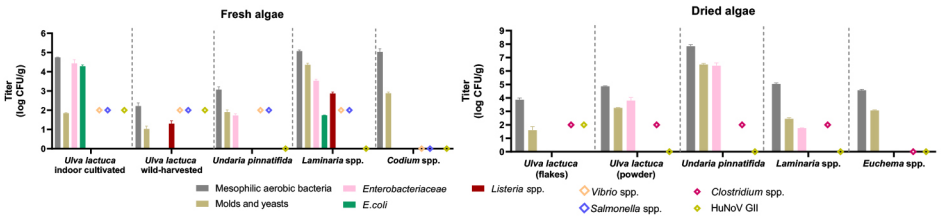


**Figure 1**. Foodborne bacterial and viral pathogens in fresh and dried edible algae. Bars represent quantifiable determinations (mean ± SE), while diamonds represent unquantifiable determinations (presence/absence).

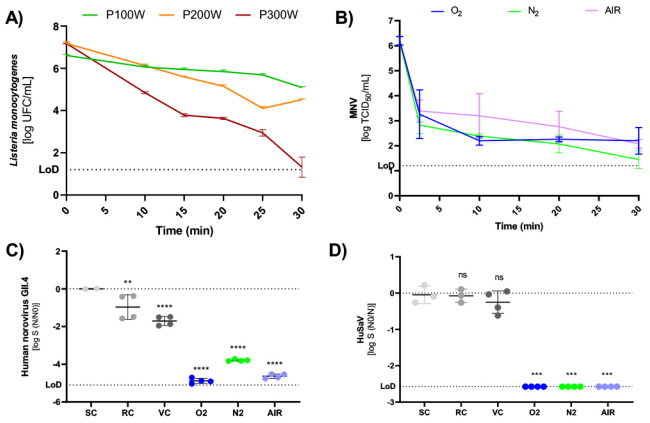


**Figure 2**. Assessment of the inactivation kinetics of CP treatments for *Listera monocytogens* (panel A) and MNV (B), and validation against HuNoV (C) and HuSaV (D) in contaminated kombu. Operating conditions included exposure time, power (100, 200, and 300 W), and gas composition (oxygen, $O_2$; nitrogen, $N_2$; or air, AIR). Experiments included solution (SC), recovery (RC), and vacuum (VC) controls.

## Conclusion

The study reports bacterial and viral pathogen contamination on edible algae, raising concerns about the microbiological risk for consumers. To guarantee food safety, cold plasma was demonstrated as an effective technology for pathogen inactivation at specific operating conditions. Overall, these results support the cold plasma technology for enhancing the food safety of marine products.

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# PHYSIOLOGICAL RESPONSES OF *Pinna rudis* TO DIFFERENT EXPERIMENTAL DIETS: A STEP TOWARD CAPTIVE CULTURE OF *Pinna nobilis*

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## Introduction

In recent years, *Pinna nobilis*, an endemic species of the Mediterranean Sea with high ecological value, has suffered mass mortality mainly due to the protozoan *Haplosporidium pinnae*, in combination with other pathogens (Carella et al., 2023). The species is currently critically endangered, with remanent populations restricted to a few protected coastal environments such as lagoons and estuaries (sanctuary areas), where salinity conditions appear to limit the progression of the disease (Katsanevakis et al., 2022). However, even these remaining populations are showing alarming declines in individual numbers and absence of recruitment (García-March et al., 2019; Nebot-Colomer et al., 2024).

Given this situation, the development of captive breeding protocols is considered a key tool for the conservation of the species. Nevertheless, all attempts made so far have failed to maintain *P. nobilis* specimens in good physiological condition over the long term or to induce successful reproduction (Hernandis et al., 2023). Among the main limiting factors in this context, diet—both in quantity and quality—plays a critical role. In particular, the type of diet provided can significantly affect the nutritional and physiological status of filter-feeding bivalves.

With the aim of improve the feeding strategies for captive culture, an experiment was conducted with juveniles of *Pinna rudis*, a protected species phylogenetically close to *P. nobilis*, to evaluate the physiological and growth responses of *P. rudis* to different experimental diets. This approach represents a necessary step toward the implementation of aquaculture-based strategies for the recovery of *P. nobilis*.

## Material and methods

Juveniles of *Pinna rudis* (11–55 mm) fixed to the ropes of fish aquaculture farms (Villajoyosa, SE, Spain) were obtained and acclimated for two weeks in open-flow aquaria under controlled salinity (37 – 38 PSU). During acclimation, individuals were fed a control diet of *Tisochrysis lutea* and *Chaetoceros neogracilis*, the FAO-recommended microalgae mix for bivalve culture. The experimental system consisted of five flow-through chamber (FTC) units (8 chambers each), with 7 chambers per unit housing two individuals each, and 1 blank chamber without animals. All experimental diets consisted on the microalgae FAO-mix, in which one portion was replaced by the following microalgae: (1) control diet (FAO-mix without replacement, C), (2) carbohydrate-rich (with *Rhodomonas lens*, RL), (3) eicosapentaeinoic acid (EPA)-rich (with *Tetraselmis suecica*, TS), (4) lipid-rich (with *Diacronema lutheri,* DL), and (5) high in inorganic matter (with added kaolin, K). Antibacterial activity was tested on each microalgae by measuring the inhibition of growth of fish pathogenic bacteria *Vibrio harveyi* (Vh) as previously described (García Beltrán et al. 2018). After one month under experimental conditions, physiological measurements were conducted, including clearance rate, absorption efficiency, respiration rate, and feces collection, to assess energy balance (S*cope for Growth*). At the end of the trial, individuals were dissected, and biometric measurements were taken to assess growth and standardize physiological rates. Tissue samples were also collected for further molecular analyses. All samplings were performed under permissions of competent authorities (SGBTM/BDM/AUTSPP/12/2024 and SGBTM/BDM/AUTSPP/12bis/2024).

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**Results and conclusions**

Among studied dietary treatments no significant differences in growth of individuals were observed. After one month under experimental conditions, individuals reached an average shell length of 70.7 ± 6.1 mm and a live weight of 5.6 ± 1.0 g. Clearance rates ranged between 1.2 and 2.3 $L \cdot ind^{-1} \cdot h^{-1}$ across all groups. However, significant differences were detected in absorption rate and absorption efficiency, both of which were lower in the group fed the *DL*–enriched diet. This reduced absorption capacity negatively impacted energy balance (S*cope for Growth*), resulting in a slight, though not statistically significant, reduction in growth. These results suggest that diet composition, particularly lipid enrichment, can affect physiological performance in *Pinna rudis*, and highlight the importance of optimizing feed formulations for successful captive rearing protocols applicable to *Pinna nobilis* conservation efforts.

The highest antimicrobial activity was recorded in the cell fraction of the microalgae cultures. The RL species shows an activity twice as high as that recorded in the other microalgae. In addition, the soluble fraction of the TS microalga exhibited the highest antimicrobial activity. Although these results do not correlate with the observed physiological differences, the higher antimicrobial potential of RL and TS adds value to these microalgae as components of the diet.

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# ENHANCING SEAHORSE HIPPOCAMPUS ERECTUS PRODUCTION IN AQUACULTURE

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## Introduction

Seahorses are unique marine fish species renowned for their distinctive morphology and reproductive behaviour. Due to their high commercial value and conservation concerns, seahorse aquaculture has gained attention as a sustainable alternative to wild collection. However, the successful maintenance and cultivation of seahorses, especially during their early life stages, presents numerous challenges. This study at CEPAM-ECOMARE (University of Aveiro, Portugal) aims to investigate key factors in the rearing of seahorses, namely *Hippocampus erectus,* including water quality, food supply, and tank management, to improve survival and optimize commercial production.

## Materials and Methods

The production of *Hippocampus erectus* at CEPAM-ECOMARE is performed in greenhouses, utilizing a 10,000 L Recirculating Aquaculture System (RAS). The seahorses are maintained in standard 200 L tanks, which are part of the RAS for efficient water circulation and optimal conditions for their growth and development. These tanks are equipped with aeration and water flow to create circular currents that enhance the distribution of *Artemia* sp. and prevent biofilm buildup. Tanks are cleaned regularly, with a standard disinfection protocol every 15 days or following abnormal mortality events. Water quality is monitored weekly, with natural seawater sourced from the adjacent coastal lagoon Ria de Aveiro (Portugal). For seahorse offspring, *Artemia* sp. metanauplii, enriched with omega-3 polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA), are provided as primary food source to support early-stage development. As seahorses transition to juvenile and adult stages, they are gradually weaned onto frozen mysids (collected from the adjacent coastal lagoon), with commercial-size adults being fed with commercial frozen mysis. Seahorse offspring are placed in a single tank, and the sizesorting process begins after the 80$^{th}$ day to optimize population distribution.

## Results

Throughout the 7-month study, 18 hatching events occurred from two breeding pairs, resulting in a total of 2,942 newly born seahorses. From these, 11 batches of *Hippocampus erectus* were reared up to 80 days post-hatching, with survival ranging from 63% to 98%. The average survival across all batches was 88%, indicating a strong success in early-stage rearing. Mortality did not follow a consistent pattern, with large periods of no mortality followed by occasional mortality events. These results are particularly promising given the typically low survival associated with early-stage seahorse rearing, which is often under 30%. After hatching, seahorses are kept at a maximum population density of 1.5 individuals per L. After the 80$^{th}$ day, sorting is carried out to control the population density, ensuring it does not exceed one seahorse per L. Once seahorses reach an adult size (> 60 mm in total length), density is reduced to one seahorse per 2 L.

## Discussion

The high survival achieved in *Hippocampus erectus* aquaculture can be explained by the use of natural seawater, which, unlike what is commonly reported, did not result in significant health issues. As such, the composition of natural seawater may be more suitable than artificial seawater when it comes to seahorse production. Additionally, the management of food supply, particularly *Artemia* sp. metanauplii enriched in DHA, proved essential to support the early-stage development of seahorse offspring. The success of the simple tank system, with modifications concerning aeration and water flow, further suggests that advanced seahorse offspring rearing tanks may not be necessary to successfully culture this seahorse species. Population management, namely through sizesorting, helped to maintain stability in the tanks and a stress-free environment. Overall, these results highlight the importance of managing population density for successful seahorse rearing.

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**Figure 1:** Production systems and developmental stages of *Hippocampus erectus*. a) Recirculating Aquaculture System (RAS) in the greenhouses at CEPAM-ECOMARE, University of Aveiro; b) Adult male *Hippocampus erectus* carrying embryos in the brood pouch; c) Early-stage *Hippocampus erectus* juvenile (2-3 weeks old); and d) *Hippocampus erectus* juveniles (4 months old), feeding on frozen mysis.

## Conclusion

The rearing of *Hippocampus erectus* has provided valuable insights into effective aquaculture practices, which are worth being tested with other seahorse species. The high survival recorded highlights the potential for commercial-scale production of seahorses, provided that key factors, such as food supply, water quality, and stocking density, are carefully managed. Future research will focus on optimizing these parameters for even larger-scale operations and explore the potential of farmed seahorses for high-end uses.

## Acknowledgements

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# ABSOLUTE MONOCULAR DEBTH ESTIMATION FOR BIOMASS PREDICTION IN AQUACULTURE

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**Introduction**

Accurate fish biomass estimation is vital for sustainable and efficient aquaculture. Traditional methods (manual measurement or stereo cameras) are expensive, invasive, or complex. Monocular cameras offer a low-cost, widely available alternative, but they typically produce relative depth maps. This project develops an AI-based system to estimate absolute depth and fish biomass using monocular images, aiming to make biomass estimation more scalable and cost-effective in real aquaculture environments.

**Objectives**

Develop a monocular camera-based system to estimate fish biomass accurately in aquaculture environments. Leverage AI techniques to convert relative depth from monocular cameras into absolute depth. Reduce reliance on expensive stereo camera systems, enabling scalable and cost-efficient biomass monitoring.

**Methodology**

- Task 1: Depth Scaling.
  - Developed scaling models (linear, quadratic, cubic) using:
    - Office environment with known object positions.
    - Underwater aquaculture environment with a reference tile.
  - Applied offline and online scaling methods:
    - Offline: Derived a fixed scale from calibration data.
    - Online: Adjusted scaling dynamically during processing.
- Task 2: Fine-Tuning Monocular Model
  - Used Depth Anything V2 model.
  - Fine-tuned with 15,000 stereo camera frames from aquaculture cages.
  - Applied masks and depth constraints (1–2m) to focus on foreground fish.

  Output: SL (Standard Length) and biomass prediction derived from scaled/fine-tuned monocular depth.

**Results**

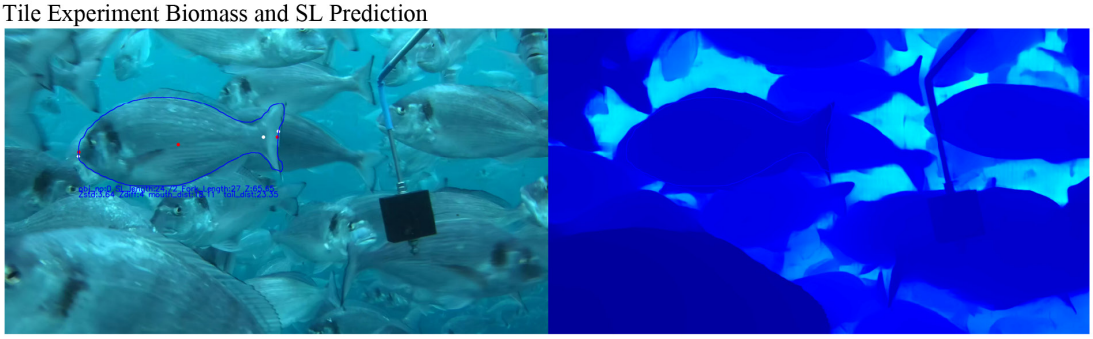
- Task 1: Depth Scaling
  - Office Environment (Fake Fish Test)
    - Best model: Cubic regression
    - Achieved SL error: 1.78 cm
  - Highest accuracy in controlled settings
  - Underwater Environment (Tile Scaling)
    - Offline scaling: More accurate but less adaptive
    - Online scaling: More consistent in changing conditions
    - After filtering for shallow depths (<90 cm):
      - SL MAE: 2.76 cm (offline) vs. 3.19 cm (online)
      - Depth MAE: 6.84 mm (offline) vs. 7.65 mm (online)
- Task 2: Fine-Tuning Depth Anything V2
  - Best model: Model_v2 (max depth = 1m)
  - Achieved:
    - SL MAE: 1.48 cm
    - Depth MAE: 3.94 mm
    - Biomass prediction within 8% of ground truth
  - Performed well across multiple aquaculture cages

*(Continued on next page)*

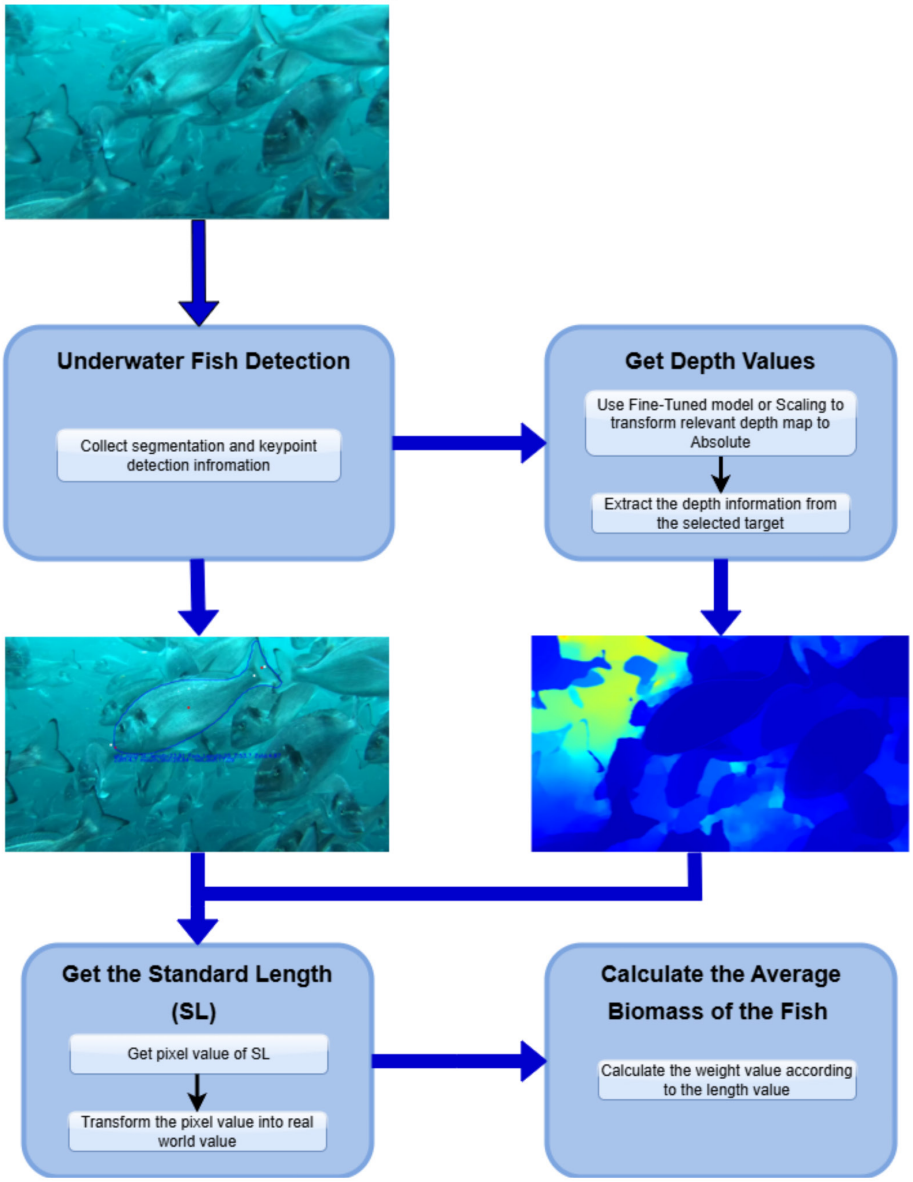
**Conclusion**

Monocular cameras, when properly scaled or fine-tuned, can achieve biomass estimation accuracy comparable to stereo systems. The tile-based scaling method (especially offline) provided a practical solution for real aquaculture conditions, with strong results after filtering for shallow depths. Fine-tuning the Depth Anything V2 model with domain-specific stereo data significantly improved accuracy, with SL and biomass errors consistently below 2 cm / 10%. The best-performing model (1m max depth) proved to be both accurate and relatively robust across different aquaculture cages. This work shows strong potential to replace expensive, high-maintenance stereo camera systems with scalable monocular setups.

Tile Experiment Biomass and SL Prediction



The flowchart of fish biomass estimation

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Task 1: SL and Biomass Results -Underwater Experiments (Tile Scaling)

| Scaling method | Predicted Average Biomass (g) | Predicted Average SL (cm) | Predicted SL STD (cm) |
|---|---|---|---|
| Offline Scaling | 500.94 | 25.90 | **3.09** |
| Online Scaling | **471.00** | **25.40** | 3.95 |
| Ground truth | 421.58 | 24.52 | 2.33 |

Task 1: SL and Depth Error for Predicted Depth Values < 90

| Scaling method | SL MAE | SL Error STD | Depth MAE | Depth Error STD |
|---|---|---|---|---|
| Offline Scaling | **2.76** | 2.97 | **6.84** | 7.26 |
| Online Scaling | 3.19 | **2.56** | 7.65 | **6.17** |

Task 2: Model Performance Comparison on SL and Biomass

| Model | Max Depth | Train Loss | d1 | AbsRel | RMSE | MAE | SiLog |
|---|---|---|---|---|---|---|---|
| Model_v1 | 5(baseline) | 0.22 | 0.76 | 0.16 | 0.44 | 0.24 | 0.25 |
| Model_v2 | **1** | **0.09** | **0.92** | **0.09** | **0.091** | **0.07** | **0.13** |
| Model_v3 | 2 | 0.15 | 0.83 | 0.13 | 0.19 | 0.12 | 0.19 |

Task 2: Models' Depth and SL error

| Model | Max depth | Average Biomass (g) | SL (cm) | SL Std (cm) |
|---|---|---|---|---|
| Model_v1 | 5 (baseline) | 440.02 | 24.86 | 3.06 |
| Model_v2 | **1** | **358.14** | **23.29** | **4.89** |
| Model_v3 | 2 | 475.46 | 25.47 | 3.05 |
| Ground Trith | - | 392.82 | 23.98 | 2.39 |

Overall Results: Testing Best Model on Different Cages

| Test Set | Model_v2 (SL, cm) | Ground Truth (SL, cm) | Mean SL Error (cm) |
|---|---|---|---|
| Same cage | 23.29 | 23.98 | 0.69 |
| Cage 15p | 26.30 | 25.16 | 1.14 |
| Cage 15p second half | 25.18 | 24.91 | 0.27 |
| Cage M7 | 24.74 | 24.61 | 0.13 |
| Cage M10 | 24.06 | 25.43 | 1.37 |

# TOXIC METAL CONTAMINATION IN FRESHWATER AND BRACKISH WATER PRAWNS (*Macrobrachium rosenbergii*) FROM SOUTHWESTERN BANGLADESH: ECOSYSTEM EXPOSURE AND HUMAN HEALTH RISK ASSESSMENT

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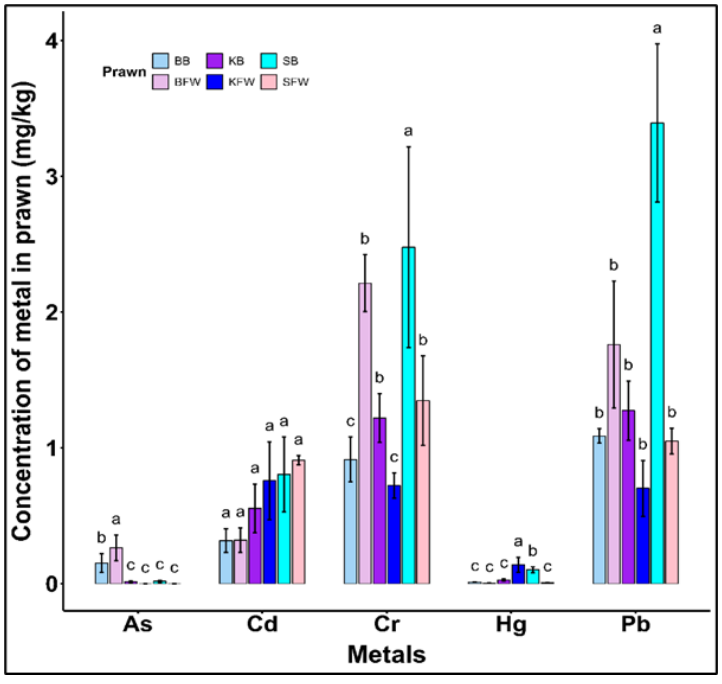
Bangladesh is acknowledged as a prominent nation in fish and shellfish production, attributed to its distinctive geographical features, including extensive rivers, wetlands, and a lengthy coastline, which create a conducive habitat for various fish species, thereby supporting numerous fisheries that constitute a vital sector of the Bangladeshi economy. Bangladesh is acknowledged as a leading country in global aquaculture, particularly in shellfish production. Prawns (*Macrobrachium rosenbergii*), often referred to as 'white gold,' are a key driver of this sector. However, rising global concerns over heavy metal contamination can be a threat to this sector.

To address these issues, we analyzed five toxic metals (As, Cd, Cr, Hg, Pb) in prawns, water, and sediment samples using the AAS method from freshwater and brackish water zones in Bagerhat, Khulna, and Satkhira district of Bangladesh. Statistical analysis revealed no significant differences (P>0.05) in toxic metal concentrations in prawns when comparing tissue types (CT vs. MU), salinity (freshwater vs. brackish water), or sampling zones (Bagerhat, Khulna, and Satkhira). Except As and Hg, all trace metals exceeded the maximum permissible limits proposed by different authorities. In the case of sediment, only Cr and Hg exceeded the allowable limits, where Pb was totally absent in water and less polluted from prawn and sediments. The BAF (2.031-76.667) was contained far below the threshold, where the BSAF values (0.014-10.2) suggested that metal actively bioaccumulated from sediment. Sediment samples exhibited the highest Metal Pollution Index (MPI) values (1.0-1.8), compared to prawn (0.14-0.8) and water (0.02-0.22). The extent of sediment contamination was assessed through the analysis of several indices, including the Pollution Load Index (PLI), Contamination Factor (CF), modified Contamination Degree (mCd), Ecological Risk Index (RI), Geoaccumulation index (), and Toxic Risk Index (TRI). No health risks were identified for either adult or child populations from the Estimated Daily Intake (EDI), Non-carcinogenic Risk (THQ), and Cancer Risk (CR) assessments. While the study suggests no immediate health hazard, it indicates that government and non-government institutions should ensure continued monitoring of metal toxicity and take proactive measures to prevent contamination in the future.This research provided an adequate overview of the heavy metal contamination in different environmental matrices, although further study is necessary to design the conceptual framework model for heavy metal contamination in an aquaculture field to better mitigate the hazards and risks in sustainable aquaculture



# HIGH ENVIRONMENTAL AMMONIA REGULATES THE EARLY LIFE DEVELOPMENT OF ATLANTIC SALMON (*Salmo salar*)

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## Introduction

The mechanisms of ammonia excretion and its associated toxicity have been extensively studied in freshwater fish; however, comparable investigations in anadromous species, particularly during early developmental stages of Atlantic salmon (*Salmo salar*), remain limited. Elevated environmental ammonia is highly toxic to fish, disrupting critical physiological processes such as osmoregulation, respiration, metabolism, neurotransmission, and cellular function, which can compromise health and even lead to mortality. However, exposure to high environmental ammonia during embryonic development may induce phenotypic plasticity, potentially conferring long-term benefits for stress resilience later in life. This study aimed to elucidate the patterns of ammonia transport in Atlantic salmon embryos and larvae up to 976 day-degrees (DD) post-fertilization. A secondary objective was to characterize the mRNA expression of key ammonia transporters (Rhag, Rhbg, and Rhcg), the urea transporter (UT), H+-ATPase, Na+/K+-ATPase, and sodium-hydrogen exchangers (NHE3 and NHE2) during early development under exposure to three sub-lethal ammonia concentrations. In addition, the study investigated whether ammonia exposure affects the cellular stress response by analyzing the mRNA expression of heat shock proteins HSP70 and HSP90.

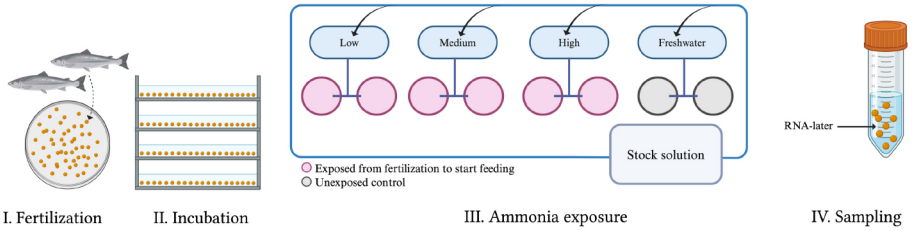
## Materials and methods

An ammonia exposure trial was conducted by fertilising a batch of Atlantic salmon eggs followed by the exposure to three levels of ammonia (low: 22 µg/L, medium: 75 µg/L, and high: 148 µg/L) until the stage prior to start feeding (976 DD). Samples were taken from six developmental timepoints for mRNA expression analysis of important genes responsible for ammonia transport, urea excretion and cellular stress response. The spatial localization of ammonia transporters was also examined at two key developmental stages: hatching (525 DD) and near complete yolk absorption (900 DD), using immunohistochemistry.

## Results and Discussion

The results demonstrated that Atlantic salmon embryos are primarily ammoniotelic throughout development, with ammonia serving as the predominant nitrogenous waste product. The mRNA expression of Rhesus glycoprotein (Rh) genes, particularly Rhag and Rhcg, as well as the UT gene, showed strong alignment with the ontogeny of ammonia and urea excretion rates observed in other salmonid species. However, exposure to elevated ammonia levels significantly altered the expression patterns of these transporters suggesting that environmental ammonia influences the regulation of nitrogenous waste excretion.

Spatial localization revealed that Rhag was expressed in the developing gills, yolk sac, and operculum, while Rhbg was localized to the gills and oral cavity. Rhcg was detected in the sub-operculum and skin. These findings indicate that ammonia excretion is the primary mechanism of nitrogenous waste elimination during early development in Atlantic salmon, with Rhesus glycoproteins playing a pivotal role in ammonia transport via the gills and yolk sac. This process is likely facilitated by the coordinated activity of H+-ATPase, Na+/K+-ATPase, and sodium-hydrogen exchangers.

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Furthermore, the upregulation of HSP70 and HSP90 in response to elevated ammonia concentrations suggests that ammonia exposure induces a cellular stress response during early development. This highlights the potential physiological impacts of environmental ammonia on developing salmon and underscores the importance of understanding how early exposure shapes stress-related molecular pathways.

**Conclusion**

This study reveals that elevated environmental ammonia regulates the early development of ammonia transport and excretion mechanisms in Atlantic salmon. Early exposure to ammonia alters the molecular responses of developing embryos, potentially influencing their ability to handle stress later in life. These findings provide valuable insights into the physiological and molecular adaptations of Atlantic salmon to environmental stressors during critical developmental stages.

**Acknowledgements**

This work was supported by the Norwegian Research Council No. 331892 (CandRAS).

# REPRODUCTIVE BIOLOGY AND OFFSPRING DEVELOPMENT SUCCESS IN FARMED COD ARE AFFECTED BY NUTRITION, LIGHT AND SUBMERGENCE

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**Introduction**

Interest in cod farming is once again growing in Norway, driven by a new generation of domesticated cod selected for improved growth through breeding programs. Despite these advancements, several challenges remain—one of the most pressing being the control of sexual maturation, which can compromise growth performance, fish health, and pose environmental risks. Indeed, in cod (*Gadus morhua*) farming, fish are typically reared in open sea cages. In case of individuals reaching maturity, they may release fertilized eggs either during a spawning event occurring in the cages or after escaping. This could result in a possible interaction with wild cod and genetic pollution of the local cod stocks in Norway. One proposed mitigation strategy is the use of continuous light (LL), or LL and shading of natural light (at surface or using submergence) which is known to delay maturation of cod in cages. Additionally, the use of standard feeds aimed at maximizing somatic growth may influence reproductive output in maturing fish when compared to broodstock diets. However, the extent to which these effects persist in the current domesticated cod strains—now in their 6th to 8th generation—remains unclear.

**Methods and setup**

To improve control of sexual maturation in farmed cod through light and submergence strategies, we employed surface cages exposed to strong continuous light (LL, 2500 W) and submerged sea cages positioned at 30 m depth (away from strong natural daylight), also under LL conditions (600 W). Additionally, two dietary treatments - broodstock (Vitalis Prima, Skretting) and standard (Amber Neptun, Skretting) diets - were tested under natural light (NL) conditions. For each treatment group, 240 to 480 cod with an average weight of 1.5 to 2 kg were stocked in cages in September 2024. LL-fish were held under continuous light in indoor tanks before transfer while NL-fish were kept under natural photoperiod. Fish from each group were sampled every six weeks for growth measurements, blood and tissue collection, and assessment of sexual maturation through various biological indicators. Parasite prevalence was also monitored, and a welfare score was developed to assess overall fish health. At the onset of the spawning season, mature fish were transferred to indoor tanks equipped with egg collectors, using a male-to-female ratio of 2:1. As a reference, wild cod captured near the cages were maintained in a separate tank under similar conditions. Egg volume and fertilization rates were recorded daily. Additionally, newly fertilized eggs were sorted and incubated in NUNC plates at 6 °C to monitor survival until hatching, determine hatching success and evaluate larval morphometry at hatch.

**Results and discussion**

At 2.5 years old, fish reached an average weight of 3.7 kg with no significant differences observed between dietary treatments. Preliminary welfare assessments - based on indicators established for salmon - suggest that the welfare of farmed cod was generally acceptable, despite the occurrence of some skeletal deformities, particularly in the backbone and head regions. Consistent with previous trials, parasite prevalence, specifically *Caligus*, was higher in LL-fish held in submerged cages. Sexual maturation was first detected in NL-individuals, while those in LL treatments entered early stages of maturation 5 months later. Both males and females underwent maturation across treatments.

Egg production during the spring spawning season (NL treatment) varied, with females fed the broodstock diet producing a greater egg volume compared to those on standard growth diets. Fertilization rates were lower and more variable in farmed cod than in the wild reference group. Survival and hatching rates were also treatment-dependent. Overall, reproductive output and developmental success were superior in wild cod compared to farmed groups. Within farmed treatments, eggs from broodstock-fed fish outperformed those from standard-fed fish, which showed poorer fertilization, survival, and developmental outcomes.

Our results indicate that both light regime and diet significantly influence the reproductive performance and developmental success of farmed cod. Continuous light effectively delayed sexual maturation, while standard diets had a negative impact on egg quality and larval outcomes compared to broodstock diets. Nonetheless, a substantial proportion of larvae survived post-hatching across treatments, suggesting that accidental spawning in cages—or following escape events—could contribute to the presence of farmed-origin individuals in the wild.

# GENOMIC ADMIXTURE AND LOCAL THERMAL ADAPTATION SHAPE LARVAL SURVIVAL IN EUROPEAN SEA BASS: IMPLICATIONS FOR SELECTIVE BREEDING UNDER CLIMATE CHANGE

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## Introduction

The European sea bass (Dicentrarchus labrax) is a key species of Mediterranean aquaculture and has undergone rapid domestication and selective breeding in the past two decades (Vandeputte et al., 2019). European sea bass breeding programmes are based on genetic resources gathered from three remarkable populations, Atlantic (AT), Western Mediterranean (WM), and Eastern Mediterranean (EM), formed through historical isolation, from which emerged two ancestral lineages (Atlantic - ATL, and Mediterranean - MED), followed by a secondary contact (Duranton et al., 2018). These populations characterized by a gradient genomic introgression between the two ancestral lineages, are differentially adapted to regional temperatures. Such introgression variation may impact larval fitness and complicate selective breeding strategies under shifting climatic conditions.

## Materials and Methods

Using a common garden design, we reared ~57,600 larvae from AT, WM, and EM origins under four thermal regimes (*rAT*, *rWM*, *rEM*, and *rAQUA*) mimicking natural and hatchery environments. Over 6,200 juveniles were genotyped with a 57K SNP array (Griot et al., 2021)identification of genomic regions involved in the resistance could improve the efficiency of selective breeding. The aim of this study was to identify quantitative trait loci (QTL, and ancestry was reconstructed via ADMIXTURE (Alexander et al., 2009)derived from multi-locus genotype data, can be used to perform a statistical correction for population stratification. One popular technique for estimation of ancestry is the model-based approach embodied by the widely applied program structure. Another approach, implemented in the program EIGENSTRAT, relies on Principal Component Analysis rather than model-based estimation and does not directly deliver admixture fractions. EIGENSTRAT has gained in popularity in part owing to its remarkable speed in comparison to structure. We present a new algorithm and a program, ADMIXTURE, for model-based estimation of ancestry in unrelated individuals. ADMIXTURE adopts the likelihood model embedded in structure. However, ADMIXTURE runs considerably faster, solving problems in minutes that take structure hours. In many of our experiments, we have found that ADMIXTURE is almost as fast as EIGENSTRAT. The runtime improvements of ADMIXTURE rely on a fast block relaxation scheme using sequential quadratic programming for block updates, coupled with a novel quasi-Newton acceleration of convergence. Our algorithm also runs faster and with greater accuracy than the implementation of an Expectation-Maximization (EM and APIS (Griot et al., 2020). Parental ATL ancestry was used to define high vs. low introgression groups (POP+ / POP−). Larval survival was analyzed using generalized linear and logistic models, including quadratic regression to estimate temperature optima.

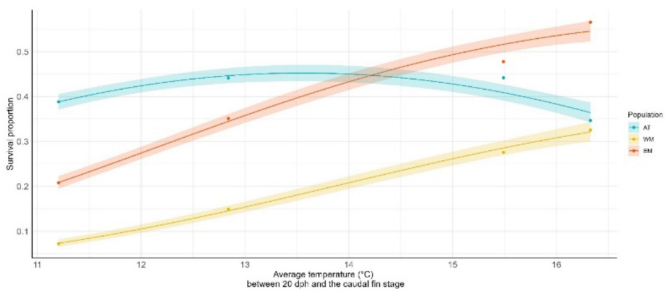


**Fig 1.** Fig. 1. Larval survival as a function of temperature in three European sea bass populations. Lines show logistic regression fits of survival rates to average temperature between 20 dph and caudal fin stage. Shaded areas: 95% CI. AT = Atlantic, WM = Western Mediterranean, EM = Eastern Mediterranean.

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**Results and Discussion**

The interaction between thermal regime and population origin had a highly significant effect on larval survival ($P < 2.2 \times 10^{-16}$), highlighting local thermal adaptation and genetic divergence among the three sea bass populations. AT larvae showed the highest survival in colder regimes (rAT and rWM), with an estimated thermal optimum of 13.6°C (Fig. 1). Conversely, EM larvae performed best in warmer regimes (rEM and rAQUA), with predicted optima near or above 17.8°C. These dome-shaped thermal reaction norms suggest divergent selection histories aligned with regional spawning periods and sea surface temperature profiles.

WM larvae consistently had the lowest survival across all regimes, with no clear optimum, likely due to high and heterogeneous genomic admixture causing outbreeding depression. These differences are unlikely to stem from egg size, larval length, or prey preference, and instead point toward disrupted genomic architecture or hybrid incompatibilities.

Introgression from the alternate lineage significantly impacted survival in both AT and EM populations. In AT, offspring from less introgressed parents (POP−, lower MED ancestry) had higher survival, especially under colder conditions. Similarly, EM larvae with lower Atlantic (ATL) ancestry survived better in warm regimes. Chi-squared tests and ancestry comparisons confirmed purging of the minor lineage, MED in AT and ATL in EM, highlighting directional selection against introgressed genomes and reduced hybrid fitness.

In WM, no such purging was detected, likely due to genome-wide saturation with introgressed tracts. This persistent admixture may prevent the elimination of maladaptive alleles, explaining WM's poor survival even under its native thermal regime. These patterns align with previous studies reporting purifying selection against Atlantic ancestry in Mediterranean populations.

# SIMULATING GROWTH AND SIZE VARIABILITY OF *Sepia officinalis* HATCHLINGS FROM DATA ABOUT ZOOTECHNICAL VARIABLES AND BIOENERGETICS UNDER CAPTIVITY

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**Introduction**

The cephalopod species *Sepia officinalis* has been extensively investigated in relation to growth under captivity. Data on size, ingestion rate, conversion rate, bioenergetics and survival are particularly abundant for the first feeding period. The present work aims to integrate published data (Grigoriou and Richardson, 2008, 2009; Bureau et al., 2002, Domingues et al., 2001a) on: size at hatching, bioenergetics, and ingestion rate, into a stochastic model to understand the effect of the initial distribution of body sizes on the temporal pattern of body size dispersion during the first feeding period of *S. officinalis*.

**Materials and methods**

The stochastic model simulated a growth experiment with 15 groups (or tanks), each group with 30 cultured hatchlings reared during the first feeding period (20 days) in an Excel spreadsheet. The experimental factor was the initial coefficient of variation of body wet weights ($CV_{WW0}$) of the group. Three factor levels of $CV_{WW0}$ (10, 20 and 30 %) were replicated five times (five groups). For each group, initial body wet weights (WW) were extracted from a normal distribution with mean 0.1 g, and one of the $CV_{WW0}$ levels. Three dependent variables were calculated for each group: i) the mean specific growth rate (SGR), ii) the mean $CV_{WW}$, and iii) the final $CV_{WW}$. Temperature, ingestion rate (IR%) and prey were based on Domingues et al. (2001a). Temperature was set at 27.5 ºC. The IR% (under ad libitum feeding) of each individual in each culture day was extracted from a normal distribution with mean IR% = 21.259 $WW(g)^{-0.173}$, and $CV_{IR\%}$ = -0.0596 WW(g) + 0.4304. Prey was assumed to be mysids with an energy content of 19.5 J/mg of dry matter. Maintenance energy of each (resting) individual in each day was based on bioenergetic principles (Bureau, 2001), data on energy losses due to excretion and the specific dynamic action, and data on standard metabolism (Grigoriou and Richardson, 2008, 2009) which depended on body dry weight and temperature. Net conversion rate of biomass was assumed to be constant (Wells and Clarke, 1996), and it was estimated to be 0.585 from experimental data on growth, ingestion and maintenance ration in *S. officinalis* hatchlings reared at 27.5 ºC. Survival rate was set at 100 %, according to literature. To determine the increase in WW of a given individual in a given day, the IR% was first simulated. Subsequently, if energy ingestion was below maintenance energy, the energy difference was assumed to come from cuttlefish tissues, and negative growth is attained. On the contrary, if energy was above maintenance, the WW for the next day was calculated according to energy content, and biomass gross conversion rate in *S. officinalis* hatchlings. This process was iterated for each hatchling in each group until day 20. Data on SGR, mean and final CV were analyzed by one-way ANOVA tests.

**Results**

The mean SGR was 10.03 ± 0.23 % day-1 for the three treatments. $CV_{WW0}$ did not affect the mean SGR ($p=0.376$) but, as expected, it exerted a significant effect on the mean $CV_{WW}$ along the experiment ($p < 0.001$), and not so clearly in the case of $CV_{WW}$ at day 20 ($p = 0.088$). In general, the higher the $CV_{WW0}$, the higher the $CV_{WW}$ at any day, but this difference was gradually getting shorter over time.

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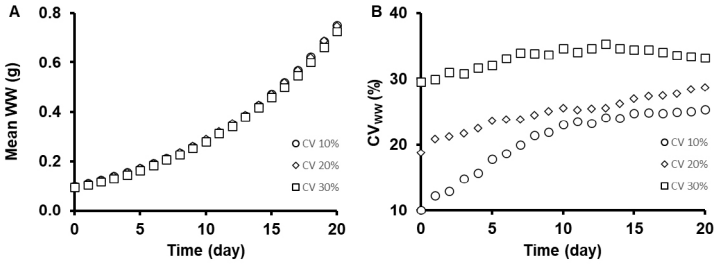


Fig. 1. Simulated growth of *S. officinalis* hatchlings. A. Mean wet weights. B. Mean $CV_{WW}$

**Discussion**

The mean SGR of 10.03 ± 0.23 % day$^{-1}$ for the three simulated treatments was reasonably close to the experimental mean SGR of 9.1 ± 3.1 % day$^{-1}$ for different individuals, reported by Domingues et al. (2001a), under similar culture conditions. A general characteristic of empirical data about growth of cuttlefish hatchlings is an increase in weight CV over time, which subsequently slows down or even changes into a $CV_{WW}$ drop (Koueta et al., 2000; Domingues et al., 2001b, 2003, 2004; Correia et al., 2008). These empirical data showed a mild positive Spearman correlation between the $CV_{WW}$ at day 0 and $CV_{WW}$ at days 10-15 despite the variations among experimental designs from different authors. The temporal pattern of $CV_{WW}$ in our simulations shows similar features, since we obtained an initial increase in $CV_{WW}$ that levels off towards the end of the first feeding period. Therefore, this work supports the usefulness of stochastic models based on zootechnical data, and bioenergetic principles to simulate body size variability in groups of cultured *S. officinalis* hatchlings.

**Acknowledgements**

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# CARACTERIZATION OF THE DAILY FEEDING RHYTHMS OF *Penaeus (melicertus) kerathurus* BY FIXED-FEEDING AND A NOVEL SELF-FEEDING SYSTEM

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**Introduction**

The remarkable growth that aquaculture has experienced in the last decade (FAO, 2024) requires the adoption of sustainable practices, with diversification through new low-trophic level species and improved feeding techniques being essential.

In Spain, despite the success of fish and mussels farming, crustacean aquaculture remains limited. Early domestications attempts were made in the 70's for caramote prawn, *Penaeus (Melicertus) kerathurus*, a high-value species in the Mediterranean, however, results were unsuccessful (Muñoz & San Feliu, 1978). Nowadays, scientific&technological advances offer new opportunities to reconsider the farming potential of caramote prawn.

This study aims to expand knowledge of *P. kerathurus* biological rhythms—key for welfare and farming efficiency (Peixoto et al., 2025)—by analyzing feeding activity rhythms under fixed-time manual feeding or a novel photocell-activated self-feeding system.

**Material and Methods**

Four *P. kerathurus* individuals obtained from local fisheries at San Carlos de la Rápita (Tarragona, Spain) were kept individually in 30 L tanks (40 x 25 x 30 cm, with 5 cm sand) with filtered, aerated water under a 13h:11h light-dark cycle (light on at 8:00 h) at the marine aquaculture laboratory of Tilamur S.L. (Lorquí, Murcia). Water quality was monitored daily (salinity, temperature, pH, and dissolved oxygen). Nitrite and nitrate were measured twice weekly (JBL PROAQUATEST COMBISET Marin) with 60% water exchanges every week.

After a one-week acclimation with daily hand-feeding (28% of tank biomass) on a raised platform, to train prawns to feed in a designated feeding zone. Two experiments were done: (1) manual feeding at 19:00 h (20 days) and (2) with self-feeding (22 days). Feeding activity was recorded using photocells and analyzed with El Temps software (v1.291) to produce actograms and calculate mean waves.

**Results & Discussion**

The recordings showed under fixed feeding, 76.3 ± 6.3% (mean ± SD) of total feeding activity occurred at night. The mean wave (Fig. 1) revealed increased feeding activity at specific times, with a first peak around 18:00 (anticipatory activity) and a larger second peak between 19:50 and 20:50 (eating activity), accounting for 68.25% of daily activity.

For the second experiment, a novel self-feeding system (Fig. 2) with a food dispenser triggered by a photocell above the feeding platform was designed to deliver food on prawn demand. To prevent overfeeding, feeding was limited to one activation every two hours during the night. Data were recorded with a custom monitoring program.

Under self-feeding, activity was mostly nocturnal (76.8 ± 9.5% (mean ± SD)), showing four peaks of activity, the biggest one at night onset (31.6 ± 1.9%). Certain cognitive capacity was observed, since they learned to operate the feeder by regulating their intake according to their biological rhythms.

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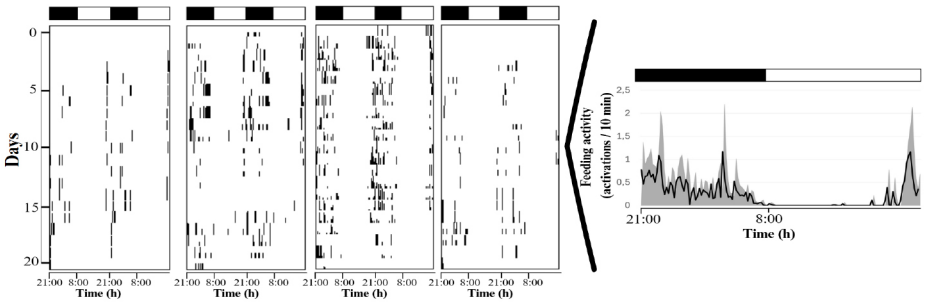


*Fig. 1Actograms (left) and mean wave plot (right) showing P. kerathurus feeding activity under fixed feeding, based on infrared beam interruptions (n = 4), with average (black) and average + standard errors (grey), represented.*

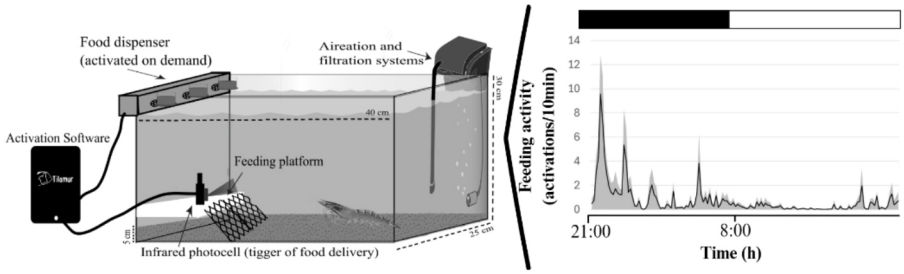


*Fig. 2 Schematics diagram of the shrimp tank equipped with the self-feeder system and mean wave plot (n=5) showing feeding activity under self-feeding.*

**Acknowledgments**

This study is part of the project 'Culture and use of marine invertebrates in aquaculture system with low environmental impact (CAMBIA)' which was founded by the Biodiversity Foundation (Spanish Ministry for Ecological Transition and the Demographic Challenge) through the Pleamar Programme, co-financed by the European Maritime, Fisheries and Aquaculture Fund (EMFAF).

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# IMPACT OF PROTEIN HYDROLYSATES ON SEROTONIN-MEDIATED GUT FUNCTION AND BARRIER INTEGRITY IN *Sparus aurata*

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## Introduction

Due to the growing demand driven by aquaculture expansion, the supply of marine-derived ingredients for aquafeeds can no longer keep pace, prompting a shift toward alternative raw materials. This transition has led to increasingly complex feed formulations, which may impact fish growth, nutrient absorption, and overall health. In this context, maintaining gut health is vital, as disruptions in gut function can impair nutrient utilization and immune function, raising the risk of disease outbreaks. To overcome these challenges, protein hydrolysates (PH), rich in essential amino acids (AAs) and bioactive peptides (<10 kDa), have emerged as promising functional ingredients in aquafeeds, providing benefits beyond their nutritional value, enhancing animal resilience. Serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter mainly produced in the gut, regulates key physiological processes, including appetite, gut motility, and mucus secretion. Certain AAs have been shown to modulating serotonergic activity, although underlying mechanisms remain unclear. Moreover, PH have shown potential in modulating intestinal physiology and integrity through the regulation of serotonin metabolism. A better understanding of serotonin metabolism, and how it can be influenced by diet, is crucial for developing effective nutritional strategies to preserve gut health, under ever evolving aquafeed formulation. Thus, this study hypothesizes that dietary PHs can improve gut physiology and barrier integrity in gilthead seabream (*Sparus aurata*) through modulation of the serotonergic metabolism.

## Material & Methods

Four diets were formulated: a commercial-like diet (CTRL) and three experimental diets (INSECT, FISH, SWINE), in which 3% of high-quality commercial PH was replaced with locally sourced agri-food-derived PHs. Juvenile fish were fed these diets thrice daily for 88 days and, at the end of the growth trial, anterior intestine samples were collected. Gut integrity was evaluated histologically using both light and transmission electron microscopy (TEM). Gene expression analyses in the anterior intestine targeted key serotonin-related genes (involved in 5-HT synthesis, transport, and receptor activity), alongside markers of gut integrity and immune response.

## Results & Discussion

All diets were well accepted, with no differences in voluntary feed intake. Fish fed the INSECT and SWINE diets exhibited significantly higher final body weight compared to CTRL. Histomorphological parameters of the anterior intestine assessed under light microscopy, such as absorption area, submucosa thickness, *villus* length, goblet cell (GC) quantification and size, revealed no significant differences among treatments. Histomorphology analyses were further supported by TEM analyses, that revealed no observable differences in the epithelial cells, presenting a regular morphology, with an intact epithelium barrier. Enterocytes showed a distinct brush border, orderly nuclear alignment, and a tall columnar morphology, and intact intercellular junctions, with no signs of disruption. Moreover, the expression of *tjp1*, a gene that encodes a tight junction protein involved in regulating the movement of macronutrients across epithelial cells, did not differ among treatments, supporting observed gut morphology and cellular architecture. Fish fed the SWINE diet showed higher expression of *slc6a4a*, a gene associated with serotonin transport. Additionally, *muc2* and *il6* were also upregulated in these fish. This suggests that an increased re-uptake of serotonin may be associated with increased mucus production and modulation of immune and inflammatory response. These findings are consistent with other animal models, where serotonin has been shown to stimulate intestinal mucin secretion. In

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contrast, INSECT fed fish also showed an upregulation of *muc2* expression, but serotonin related genes remained unaffected. Notably, despite a trend ($p = 0.054$) toward fewer GCs, which remained unchanged in size, *muc2* expression was upregulated in fish fed the INSECT and SWINE diets. Thus, this could reflect a compensatory mechanism where increased transcriptional activity helps sustain mucus production despite fewer GCs. Gene expression results will be further discussed to clarify the links between gut integrity, immune response and 5-HT system response.

**Conclusion**

This study showed that PHs derived from swine by-products and insect meal promote growth in gilthead seabream while preserving intestinal integrity. All PH supported a healthy intestinal architecture, with enterocytes maintaining normal morphology and tissue organization. Although a slight reduction in goblet cell number was observed, the concurrent upregulation of *muc2* expression in INSECT and SWINE suggests an adaptive mechanism that maintains mucosal protection and secretory function. Moreover, fish fed the SWINE diet showed higher expression of genes related with serotonin transport, suggesting a potential modulatory effect on serotonin-mediated gut functions. These findings point to a broader impact of this PH on gut homeostasis and neuroimmune communication. Collectively, these results highlight the value of PHs, particularly SWINE and INSECT, as functional ingredients in aquafeeds, with the potential to support not only growth but also gut health and integrity. Ongoing molecular analyses will further clarify the mechanisms by which PHs influence serotonin pathways and contribute to long-term gut well-being in aquaculture species.

**Acknowledgments**

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# USE OF MEALWORM (*Tenebrio molitor*) MEAL PRODUCED UNDER CIRCULAR ECONOMY PRINCIPLES: EFFECTS ON GROWTH PERFORMANCE AND NUTRIENT UTILIZATION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

Insects such as the yellow mealworm (*Tenebrio molitor*) are increasingly recognized as sustainable alternative ingredient in aquafeed production, due to their high protein content, well-balanced amino acid profile, and valuable mineral and vitamin composition [1]. Additionally, mealworm meal (MM) production has a relatively low environmental footprint [2]. However, the economic feasibility of MM production remains a challenge, prompting the exploration of cost-effective rearing strategies. One promising strategy involves incorporating agro-industrial byproducts insect rearing substrates. This strategy may reduce production costs while simultaneously influencing the yield and nutritional quality of the final product [3, 4]. Among this byproducts, spent mushroom substrate (SMS), the primary residue of mushroom cultivation, [5] has been identified as a sustainable alternative to traditional substrates like grains and seeds [6], potentially minimizing nutrient losses across the production chain.

This study aimed to evaluate the use of MM produced based on larvae partially reared on SMS, compared to conventional MM, as fishmeal replacement in juvenile rainbow trout (*Oncorhynchus mykiss*) diets.

## Materials and methods

Two different yellow MM (pre-pupae stage) were evaluated. One MM (CC-MM; protein 51.9%, lipids: 32.8%) was produced using a commercial rearing cereal-based substrate (CC), while the test MM (SMS-MM; protein 53.1%, lipids: 24.2%) was produced with a substrate composed of 80% commercial substrate and 20% SMS. Five isoproteic (45%) and isolipidic (22%) experimental diets were formulated. A control diet (CTRL) was formulated to resemble a conventional commercial trout diet, containing 20% fishmeal. Four additional diets were formulated by partially or fully replacing fishmeal with either CC-MM or SMS-MM at inclusion levels of 12.5% or 25%, corresponding to 50% or 100% of fishmeal, respectively (diets: CC12.5, CC25, SMS12.5, and SMS25). A feeding trial was conducted using juvenile rainbow trout (initial body weight: 11.4 ± 1.5g trout$^{-1}$) with 20 fish randomly assigned to each of 15 fiberglass tanks (120L) within a recirculating aquaculture system (RAS). Fish were hand-fed to apparent satiation twice daily over an eight-week period. At the end of the trial, growth performance, whole-body composition and digestive enzymes activity were evaluated. Simultaneously, a digestibility trial was conducted (42.6 ± 15.6 g trout$^{-1}$) reared in a separated RAS with 60 L capacity fiberglass tanks, each connected to a faecal sedimentation column (Guelph system), to determine the apparent digestibility coefficients (ADC) of the experimental diets

## Results

Fish fed diets containing SMS-MM exhibited significantly higher ADC for dry matter, protein, ash, phosphorus, and energy compared to those fed diets with CC-MM. Irrespective of the MM type, increasing the inclusion level from 12.5% to 25% further improved nutrient and energy digestibility. No significant differences were observed in overall growth performance among dietary treatments. However, voluntary feed intake was reduced in fish fed the SMS25 and CC25 diets compared to the CTRL group. In contrast, fish fed CC25 diet showed improved feed efficiency, higher protein efficiency ratio and a lower

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hepatosomatic index relative to those fed the CTRL diet. Whole-body lipid and energy contents were significantly higher in those fed SMS-MM-based diets compared to CC-MM-based diets. Moreover, independent of MM type, a 25% dietary inclusion led to higher whole-body lipid and energy contents and reduced ash and phosphorus contents compared to 12.5% inclusions. Regarding digestive enzymes activities, no significant differences were observed in α-amylase activity among treatments. However, fish fed 25% MM diets exhibit elevated lipase activity compared to those fed 12.5% MM diets, while fish fed SMS25 showed higher trypsin activity compared to the CTRL group.

**Conclusion**

The dose-dependent inclusion of both types of mealworm meal (MM) had no significant effect on the growth performance of juvenile rainbow trout, although feed intale was reduced at the 25% inclusion level. Mealworm meal produced using rearing substrate partially composed of spent mushroom substrate (SMS) demonstrated comparable nutritional adequacy to that produced with conventional substrates. However, the inclusion of both MM influenced nutrient utilization, with distinct effects observed on lipid deposition, digestibility, and digestive enzyme activity. These findings highlight the potential of incorporating agro-industrial byproducts into insect rearing substrates for sustainable aquafeed production, aligning with the objectives of the EU's Farm-to-Fork strategy.

**Acknowledgements**

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# CIRCULAR PRODUCTION OF MEALWORM (*Tenebrio molitor*) MEAL: EFFECTS ON BIOACTIVE PRPERTIES AND IMPLICATIONS FOR RAINBOW TROUT (*Oncorhynchus mykiss*) IMMUNE AND OXIDATIVE STATUS

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**Introduction**

The yellow mealworm (*Tenebrio molitor*), an emerging alternative ingredient for aquafeeds with potential to influence the immune and oxidative status of fish [1]. Modifying the rearing substrate used in mealworm production is a promising strategy to enhance productivity, which may modulate not only the nutritional value, but also the functional properties of the end-product [2, 3]. Spent mushroom substrate (SMS), the main waster of mushroom production, presents a sustainable alternative to conventional cereal-based rearing substrates traditionally used in mealworm farming [4, 5]. This study aimed to evaluate the impact of larval rearing substrate composition on the bioactive and functional properties of mealworm meal (MM). Additionally, the effects of dietary inclusion of both MM types on the immune and oxidative status of juvenile rainbow trout (Oncorhynchus mykiss) were evaluated.

**Materials and methods**

Two different MM were used, a commercial MM (CC-MM, crude protein 51.9%, crude lipids: 32.8%) and a teste MM (SMS-MM, crude protein 53.1%, crude lipids: 24.2%), produced using a substrate composed by 80% of commercial rearing subtrate and 20% of SMS. The bioactive and functional properties of CC-MM and SMS-CC were assessed in an *in vitro assay*. Functional compounds were extracted from both MM using three different extraction methods: distilled water, 1:1 acetone and methanol mixture, or dichloromethane. The antimicrobial activity of the crude extracts was tested against six fish-relevant reference microorganisms (*Aeromonas hydrophila* DSM 3018, *Yersinia ruckeri* ATCC 29473, *Listonella anguillarum* ATCC 19264, *Lactococcus garvieae* DSM 20684, *Tenacibaculum maritimum* ATCC 43397 and *Edwardsiella tarda* DSM 30052) using the agar-based disk diffusion method [6]. A growth trial was then conducted to determine the effect of the dietary inclusion of both MM types on the immune and oxidative status of rainbow trout. Five isoproteic (45%) and isolipidic (22%) experimental diets were formulated: a control diet (CTRL) similar to a traditional commercial trout diet and four test diets based on the CTRL diet, including 12.5% or 25% of CC-MM or SMS-MM, replacing either 50% or 100% of fishmeal, respectively (CC12.5, CC25, SMS12.5, SMS25 diets). Fifteen groups of 20 juvenile rainbow trout (11.4 ± 1.5g) were randomly distributed over 15 fiberglass tanks (120L) within a recirculating aquaculture system (RAS). Fish were fed twice daily, ad libitum, for eight weeks. Haematologic parameters, plasma metabolites, innate immune parameters, and hepatic and intestinal oxidative stress indicators were analysed.

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**Results**

The *in vitro* antimicrobial assays showed that extracts from CC-MM could inhibit the growth of *Lactococcus garvieae* and *Tenacibaculum maritimum*, while no inhibitory effect was observed in SMS-MM extracts. At the end of the growth trial, the increased inclusion level of MM from 12.5 to 25%, regardless of MM type, reduced lactate levels and alternative complement pathway activity and increased cholesterol levels in the plasma of juvenile rainbow trout. No significant effect was observed on plasma glucose or triglyceride levels, nor on lysozyme, peroxidase, protease, or anti-protease activities. Fish fed CC25 showed significantly higher hemoglobin levels compared to those fed the CTRL diet, while hematocrit values remained unaffected by dietary treatment. Hepatic and intestinal lipid peroxidation was also unchanged across experimental diets. Hepatic catalase activity was higher in fish fed 12.5% MM inclusion diets compared to those fed 25% inclusion diets, regardless of the MM type. Notably, fish fed the SMS diets demonstrated increased intestinal glutathione S-transferase activity. Fish fed the CTRL diet showed a significantly higher intestinal glutathione reductase activity than all other experimental treatments.

**Conclusion**

In the present study, CC-MM extracts showed antimicrobial activity, while the SMS-MM extracts showed no detectable effect. However, when included in aquafeeds, both CC-MM and SMS-MM had a similar impact on rainbow trout juveniles' immune and hepatic and intestinal oxidative status. These findings indicate that producing MM with a circular approach, using SMS, does not impair fish health. Further studies, including bacterial challenge trials, are recommended to assess the impact on disease resistance.

**Acknowledgements**

This work was funded by the SAFE project (SmartAqua4FuturE) within the European Union's Horizon Europe programme under grant agreement no. 101084549**.** This research was also supported by national funds through FCT (Foundation for Science and Technology), within the scope of UIDB/04423/2020, UIDP/04423/2020 and D. Amaral doctoral grant (2023.03476.BD).

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# EFFECTS OF MEALWORM (*Tenebrio molitor*) MEAL PROCESSING ON RAINBOW TROUT (*Oncorhynchus mykiss*) NUTRITIONAL VALUE

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## Introduction

Insect meals have been extensively studied as a sustainable alternative ingredient for aquafeeds, particularly the yellow mealworm (*Tenebrio molitor*). To date, most studies have focused on defatted insect meals, which contain between 4–14% lipid content [1]. Industrial processing of insect larvae often includes defatting, a thermal-mechanical fat removal step [2], due to the high susceptibility of lipids to oxidation, which may compromise shelf-life and hinder pulverization processes. Nevertheless, the defatting process may reduce both the environmental sustainability and economic viability of insect meal production and potentially affect its functional properties [3]. Notably, mealworms have a naturally high fat content, reaching 30% [1]. This study aimed to evaluate the comparative effects of full-fat and defatted mealworm meal as alternative feed ingredients to fishmeal, on growth performances, whole-body composition, fillet fatty acid profile, and quality attributes of rainbow trout (*Oncorhynchus mykiss*).

## Materials and methods

A single batch of mealworm larvae was either subjected or not subjected to a thermos-mechanical defatting process, resulting in two distinct mealworm meals (MM): a full-fat mealworm meal (FFMM, crude protein: 51.9%, crude lipids: 32.8%) and a defatted mealworm meal (DMM, crude protein: 66.7%, crude lipids: 12.9%). Three isoproteic (45%) and isolipidic (22%) experimental diets were formulated for juvenile rainbow trout. A control diet (CTRL) was formulated to mimic the composition of a conventional commercial trout diet. Two other diets were formulated based on the CTRL diet but incorporated 25% of the full-fat and defatted MMs, totally replacing fishmeal (FFMM and DMM diets). All diets included 11.5% of fish oil (dry matter basis), and rapeseed oil was adjusted to maintain consistent lipid levels across all formulations. A growth trial was conducted with juvenile rainbow trout (11.4 ± 1.5 g). Nine homogeneous groups of 20 fish each were randomly assigned to nine fiberglass tanks (120L), within a recirculating aquaculture system kept at a constant 14 °C. Fish were fed to apparent satiation, twice daily, over an eight-week period. At the end of the trial, zootechnical parameters, whole-body composition, and fillet quality and composition were evaluated.

Table 1 - Muscle fatty acid composition of the rainbow trout fed the experimental diets

| Fatty acids (% fatty acids) | CTRL | FFMM | DMM | PSEM | ANOVA |
|---|---|---|---|---|---|
| SFA | 21.3 $^{a}$ | 25.4 $^{b}$ | 21.9 $^{a}$ | *0.68* | < 0.001 |
| MUFA | 39.0 $^{b}$ | 35.8 $^{a}$ | 38.6 $^{b}$ | *0.55* | 0.005 |
| PUFA | 37.4 | 36.1 | 37.1 | *0.27* | ns |
| n-3 | 22.4 $^{c}$ | 16.9 $^{a}$ | 20.1 $^{b}$ | *0.80* | < 0.001 |
| n-6 | 14.1 $^{a}$ | 18.4 $^{c}$ | 16.3 $^{b}$ | *0.63* | < 0.001 |

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. n-3: n-3 PUFA; n-6: n-6 PUFA. Values are presented as mean and pooled standard error of the mean (PSEM). Superscript letters indicate significant differences ($p < 0.05$) between the experimental treatments

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**Results**

At the end of the feeding trial, rainbow trout exhibit an eightfold increase in body weight, with no significant difference observed in overall zootechnical parameters across dietary treatments. However, both the FFMM and DMM diets led to a reduction in feed intake, while simultaneously improving feed efficiency. Furthermore, the hepatosomatic index was significantly lower in fish fed the FFMM diet compared to fish fed the CTRL diet. Whole-body dry matter and protein contents were unaffected by experimental diet. In contrast, lipid content was significantly higher in fish fed the DMM than in those fed the CTRL diet. Additionally, whole-body energy and ash contents were significantly elevated in fish from both the FFMM and DMM groups relative to the CTRL group. In the fillet, no significant differences were observed in dry matter and protein contents, among the dietary treatments. However, fillet lipid content was higher in the FFMM group compared to the DMM group. No differences were observed in muscle lipid peroxidation, pH, or textural parameters (hardness, adhesiveness, springiness, cohesiveness, chewiness, and resilience). Colorimetric analysis showed higher b* values in the CTRL and FFMM groups compared to those from the DMM group, indicating a more yellow coloration in the former treatments. The fillet fatty acid class profile is summarized in Table 1. SFA and MUFA contents were higher in the FFMM groups compared to both the CTRL and DTM groups. The CTRL diet led to the highest concentrations of n-3 PUFA and the lowest n-6 PUFA contents, followed by DMM and FFMM diets. Consequently, fish fed the FFMM diet exhibited higher atherogenicity and thrombogenicity indexes, whereas fish from the CTRL and DTM groups demonstrated higher hypocholesterolemic / hypercholesterolemic ratios and peroxidation indexes

**Conclusion**

Overall, the defatted mealworm meal promoted a more favourable fatty acid profile, with higher n-3 and n-3/n-6 ratio, and more beneficial health-related lipid indices. These results suggest that defatted mealworm meal may be a more suitable replacement for fishmeal. However, the use of full-fat mealworm meal could also be considered to reduce the need for added vegetable oils.

**Acknowledgements**

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# THE EFFECTS OF INDUCED SWIMMING ACTIVITY ON STRESS, IMMUNE AND ANTIOXIDANT STATUS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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**Introduction**

Swimming is a fundamental behavioural element that contributes to fitness in most fish species. Promoting swimming activity in aquaculture has emerged as a strategy to enhance fish welfare and physiological resilience, particularly in flow-based systems on land, such as raceways [1]. Controlled swimming has been shown to modulate the stress axis and boost immune competence [2]. Still, the physiological outcomes depend strongly on exercise intensity and pattern, and varies greatly across species. This study investigated the short-term effects of different swimming regimes on stress response, innate immune system and antioxidant status in juvenile rainbow trout (*Oncorhynchus mykiss*), using a standardized individual flume setup.

**Materials and methods**

Thirty-two fish (body length (BL): 13.0 ± 0.2 cm) were individually induced to swim in acrylic swimming flumes for 6 hours to one of four experimental conditions: minimal water flow (control, < 0.1 $BL \cdot s^{-1}$); low-speed (L, 0.8 $BL \cdot s^{-1}$); high-speed (H, 2.3 $BL \cdot s^{-1}$); and oscillating speeds (O, 0.8/2.3 $BL \cdot s^{-1}$); n = 8. Immediately after the trials, fish were anaesthetised with MS-222 (0.1 $g \cdot L^{-1}$) and subsequently euthanised. Skin mucus was collected by carefully scraping the dorso-lateral surface of the fish [3]. Blood samples were collected immediately with heparinised syringes via the caudal puncture. The blood was centrifuged (10,000 × *g*, 5 min, 4 °C) to obtain plasma. Cortisol, glucose and lactate levels were determined in plasma, while lysozyme, peroxidase, protease and antiprotease activities were measured in both plasma and skin mucus [4]. Liver and red and white skeletal muscle were dissected for oxidative stress biomarkers [5]. Data were analysed by one-way ANOVA to determine differences between groups. The level of significance used was $P < 0.05$ for all statistical analyses.

**Results and discussion**

Rainbow trout induced to swim for 6 h in L, H, or O conditions exhibited significantly lower plasma cortisol levels compared to the non-swimming control group, indicating a reduced neuroendocrine stress response. This aligns with earlier findings in salmonids suggesting that induced swimming activity generates a decrease in cortisol release [2].

While no significant alterations were detected in plasma immune markers, skin mucus samples revealed enhanced innate immunity, as shown by elevated lysozyme and peroxidase activities in fish from the L and H groups. These enzymes are key components of the first-line mucosal defence and their up-regulation may reflect localized immune stimulation.

Red and white muscle tissues from fish in L and H conditions displayed improved redox status, with an increased reduced/oxidized glutathione ratio, suggesting enhanced antioxidant defences and improved cellular protection against reactive oxygen species (ROS). This redox shift may represent a physiological adaptation to moderate swimming-induced aerobic metabolism. In contrast, the O group showed elevated lipid peroxidation in liver, indicative of oxidative damage. The unsteady nature of oscillating flows may increase metabolic fluctuation and ROS production, thereby overwhelming hepatic antioxidant capacity. These contrasting outcomes across induced swimming conditions highlight the importance of applying the most favourable water flow towards optimisation of physiological responses.

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Overall, the findings support the hypothesis that induced swimming can activate beneficial physiological responses in a context-dependent manner, with outcomes varying by tissue type and swimming regime.

**Conclusion**

These findings highlight the potential of short-term induced swimming to modulate stress, immune, and redox responses in rainbow trout. Enhanced mucosal defence and antioxidant status, combined with reduced cortisol levels in plasma, suggest that controlled swimming may serve as a useful strategy to improve fish resilience before stressful aquaculture procedures such as handling or transport. However, the observed oxidative stress in fish under oscillating conditions underscores the need for careful protocol design. Further research under group settings and longer durations is needed to assess the long-term implications of these responses in production environments.

**Acknowledgements**

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# PRACTICAL CHALLENGES WHEN INJECTING MULTIPLE VACCINES INTO MILLIONS OF FISH

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Injection vaccines are quickly becoming the method of choice for farmed fish species kept in high densities, mainly due to the long-term protection they provide for the fish. In order to ensure proper immunization, animal welfare, and sustainability, it is important that the vaccinations are done accurately, gently and swiftly.

Today the majority of fish are vaccinated by hand, but vaccination by machine is becoming increasingly more common in salmon, trout, sea bass, and tilapia farming. Current manual injectors can inject one or two vaccines intraperitoneally or intramuscularly, but it is not possible to inject in two different locations simultaneously.

Delivering vaccines accurately and gently requires skill, and in many regions, there is a shortage of trained personnel. Machine vaccination offers a way to maintain consistency and precision, even when human resources are limited.

We will present the use of injection tools to enable vaccinations of large numbers of fish, while increasing production efficiency, improving sustainability, and maintaining a high level of fish health. We will also touch on emerging opportunities made possible by new technologies. One of the most promising is image-based data capture — vaccination day is often the only time each fish is individually handled. Could that moment also be used to gather meaningful information about the fish and its health status with the aid of Artificial Intelligence?

# AI QUANTIFICATION OF ATLANTIC SALMON BEHAVIOR FROM SINGLE POINT VIDEO TRAINED WITH AUTO LABELED SYNTHTIC DATA

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## Introduction

The problem of segmentation and pose prediction of animals in images is still a significant challenge. Part of the difficulty lies in the complexity, accuracy and volume of training data required. This work presents a novel method for creating synthetic training data. This data is used to train a convolutional neural network that can analyze salmon behavior in consecutive frames. The use of synthetic data solves the problem of data amount and accuracy and allows for model evaluation with a known ground truth.

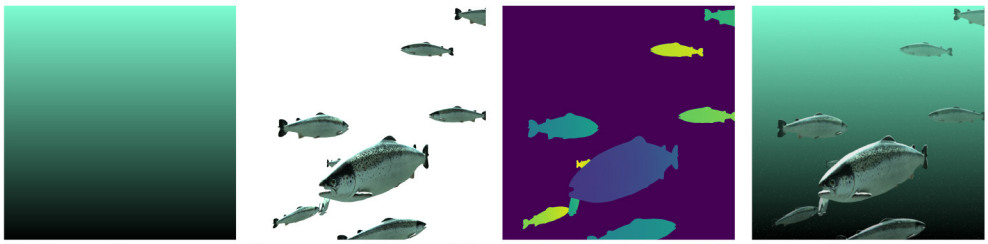
## Synthetic training data

The synthetic training data is primarily created by manipulating a single 3D model of a salmon. This 3D model is copied and given random poses within the view cone of a virtual camera along with some light sources. The image of the salmon instances is rendered and combined with a randomized gradient background. The image is completed by adding some random noise and a soft Gaussian blur. This process is illustrated in Figure 1. An arbitrary number of randomized images can then be created using this process.

The use of a known 3D object also allows for attaching key points to the model mesh. These key points can then be used to train a pose prediction model like YOLO11 pose. The model along with the key points representing a simple fish spine is shown in Figure 2.

## Initial AI model results

The synthetic training data has been used to train a YOLO11 instance segmentation model that has never seen a real salmon. The initial results from this training are promising, as can be seen in Figure 3, where the trained model is evaluated on a real video capture of salmon. This initial model can produce serviceable masks for the same individual over several frames. The positive results have prompted further research into pose estimation of salmon using key points. Research on pose estimation is currently in the works and will be further elaborated on.



*Background* | *Instances of fish model with lighting* | *Masks of fish model with occlusion* | *Composite image*

Figure 1: Decomposition of synthetic images



Figure 2: Salmon model with key points

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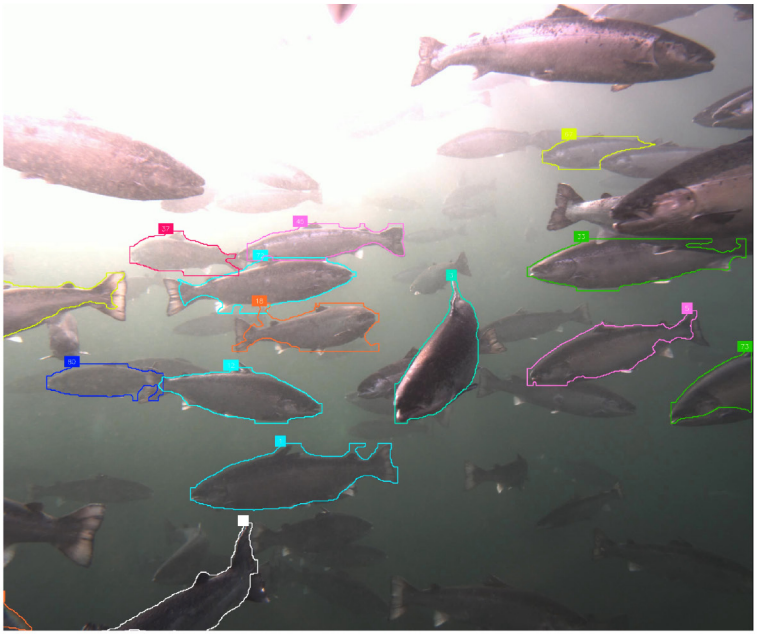

Figure 3: Instance segmented and object tracking of (real) salmon with YOLO11

# EFFECTS OF CO-FARMING RAINBOW TROUT (*Oncorhynchus mykiss*) AND DUCKWEED (*Lemna minor*) ON NITROGEN CYCLING AND FISH HEALTH IN RAS

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**Background**

Co-farming within integrated multitrophic aquaculture (IMTA) can improve the nutrient retention and utilization in aquaculture. In IMTA systems, the nutrients from fed species are consumed by extractive species, thereby converting waste products into new biomass. Duckweed (*Lemna minor*) is a freshwater plant that has been suggested as an efficient nitrogen and phosphate remediator in aquaponics and Recirculating Aquaculture Systems (RAS). The high growth rate in combination with an ability to assimilate nitrogen from both ammonia, nitrite, and nitrate, makes it a suitable for co-farming with fish. In warm-water RAS, duckweed has been found to reduce ammonia concentrations in the water and improve fish growth. There is, however, a lack of studies on the potential effects of using duckweed as an extractive species in cold-water RAS.

On the Swedish west coast, RAS are expected to be the main system for future aquaculture expansion due to strict environmental laws in combination with coastal waters in risk of eutrophication. To achieve a high degree of water recirculation in RAS, there is a need for efficient methods to utilize the dissolved nitrogenous waste. Co-farming with duckweed is a potential solution to extract the nitrogens and thereby improve system water quality. As the main farmed fish species in Sweden is rainbow trout (*Oncorhynchus mykiss*) in cold freshwater, duckweed can be added on as an extractive species without altering the preferred water parameters. The research question in this study was to evaluate the effects on water quality and system nitrogen as well as growth, health, and welfare of rainbow trout when co-farmed with duckweed. To assess this question, we performed a 6-week co-farming trial in two identical RAS systems except that one system held only fish and the other held both fish and duckweed that were farmed in a separate rack system connected in parallel to the RAS.

**Material and methods**

Two identical freshwater RAS (425 L) were set-up in a temperature control room (water temperature 12.2 °C), each containing four aquaria (93.2 L). The RAS loop contained two mechanical filters, a pump sump with a biofilter of bio balls, a UV-filter, and air bubbling. At the experimental start 72 rainbow trout, with an average mass of 47.5 ± 0.86 g (± SEM), were added to each system. One of the RAS also had a rack with four duckweed tanks (23 L) attached in the loop, with LED lights above each tank. At the experimental start, 186 g duckweed was distributed between the holding tanks. The experiment lasted for 6 weeks.

Water samples were taken three times per week to analyse for dissolved ammonium ($NH_4^+$), nitrite ($NO_2^-$), nitrate ($NO_3^-$), and phosphate ($PO_4^{2-}$). Dissolved inorganic nitrogen (DIN) was calculated as the sum of $NH_4^+$, $NO_2^-$, and $NO_3^-$. The mass of feed given to the fish was registered for each day and system and remaining feed pellets and faeces were collected and separated. The pellets and faeces were dried and weighted and sent for analysis of total nitrogen content. Duckweed was harvested when required, based on their density, and the removed biomass was weighted and sent for analysis of total nitrogen content. Selected observational welfare indicators (OWIs) were recorded for all fish triweekly, and each tank was scored for presence (1) or absence (0) of the following four OWI parameters: skin haemorrhage, scale loss, snout wound, and eye damage.

At the final sampling, fish (n = 12) were euthanized in metomidate (12 mg/L) and killed with a sharp blow to the head. A blood sample was taken from the caudal vein, centrifuged, and the plasma was stored in -80 °C for subsequent analysis of cortisol and ion concentrations. Samples of skin, proximal intestine, and distal intestine were taken for *in vitro* analysis of barrier function using the Ussing chamber technique. Liver samples were taken and placed in liquid nitrogen until analysed for two oxidative stress markers: lipid peroxidation (LPO) and glutathione assay.

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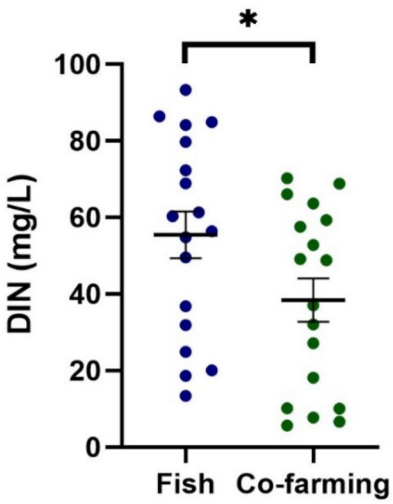


**Figure 1.** The DIN concentrations in the two s
ystems throughout the study.

| Welfare indicator | Fish | Co-farming |
|---|---|---|
| Skin hemorrhage | 0 | 0 |
| Scale loss | 70,6 | 55,9 |
| Snout wound | 70,6 | 60,3 |
| Eye damage | 2,9 | 2,9 |

**Table 1**. Score of observational welfare indicators (%).
farming system.

**Results and discussion**

DIN concentrations in the co-farming system (38.3 ± 5.5 mg/L) were lower than in the system with only fish (55.4 ± 5.9 mg/L; $t(34) = 2.049$, $p = 0.048$). This reduction was reflected in lower concentrations of $NO_3^-$ and $NO_2^-$ but not $NH_4^+$. The dissolved nitrogen was mainly present as $NO_3^-$ (54.45 ± 5.90 mg/L for fish, 37.27 ± 5.49 for co-farming) with less as $NO_2^-$ (0.59 ± 0.04 mg/L and 0.38 ± 0.04, respectively) and even less as $NH_4^+$ (0.37 ± 0.03 mg/L and 0.69 ± 0.07, respectively). This suggests that the duckweed was an efficient assimilator mainly of $NO_3^-$ and $NO_2^-$ in our system. The $PO_4^{2-}$ concentration tended to be lower, although not statistically significant, in the co-farming system (6.53 ± 1.86 mg/L) than the fish system (6.83 ± 0.80 mg/L).

The pH was higher for the co-farming system (7.24 ± 0.07) than the system with only fish (6.95 ± 0.11; $t(34) = -2.183$, $p = 0.036$). Maintaining a high pH in RAS is a challenge as it tends to be reduced by $CO_2$ production of fish and microbes at the same time as the alkalinity is consumed by nitrifying bacteria in the biofilter. The results suggest that co-farming with duckweed could help counteract these effects.

There were no growth differences of the rainbow trout between the two systems. The welfare indicators scale loss and snout wound, although not statistically significant, tended to be less frequently occurring in the co-farming system.

At the conference, remaining effects on fish health and welfare will be presented, including plasma cortisol and ion concentrations, skin and gut barrier function, and oxidative stress markers in the liver tissue.

# ACCESSIBLE DEPOSITION MODELLING TOOLS FOR IMPROVED ENVIRONMENTAL PLANNING (ADEPOPLAN)

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The overall objective of the ADepoPlan project is to improve the environmental planning toolbox available for the Faroese environment, for both industry and government, by making validated deposition models more accessible to stakeholders with limited modelling and programming experience.

As salmon aquaculture in the Faroe Islands continues to grow and expand into more exposed environments, accurate predictions of particulate organic matter (POM) deposition footprints, for both non-dispersive and dispersive fish farms, are essential for both sustainable management and regulatory compliance.

To address the need for accessible deposition models, we have developed a user-friendly prototype application that integrates the open-source Lagrangian Advection and Diffusion Model (LADiM) with 3D hydrodynamic forcing data from the FarCoast hindcast archive, developed at Firum. This R Shiny-based application incorporates a combination of Python and R code, ensuring ease of use while maintaining model flexibility. The app facilitates the integration of high-resolution oceanographic data, allowing users to simulate and predict POM deposition with minimal technical expertise. The application will be open-source at the end of the project for everyone to use and to generate trust in the model output by promoting transparency and reproducibility.

The prototype can enhance decision-making by improving site selection, impact assessments, and adaptive management strategies. Additionally, it strengthens environmental monitoring by identifying optimal locations for sediment sampling, allowing for more targeted and effective monitoring programs.

By making advanced deposition models more accessible, the ADepoPlan project strengthens environmental management of aquaculture and supports sustainable marine resource use.

# INFLUENCE OF LIGHT AND TEMPERATURE ON MATURATION AND REPRODUCTIVE SUCCESS IN *Hediste diversicolor*

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## Introduction

Sustainable growth of the salmon aquaculture industry is highly dependent on the availability of new and locally produced feed resources (Albrektsen et al., 2022, Tacon et al., 2011), and improved approaches to managing and utilizing waste streams from production (Waite et al., 2014, Turchini et al., 2019). Polychaetes can upcycle valuable biomolecules contained in aquaculture sludge and other waste streams (Anglade et al., 2023, Wang et al., 2019a), while at the same time being a potential feed ingredient in aquafeeds (Monteiro et al., 2024, Wang et al., 2019b). Future large-scale production of the polychaete *Hediste diversicolor* (OF Müller, 1776) will be dependent on extensive knowledge, control, and manipulation of the life cycle of the species (Olive, 1999, Wang, 2020). In this study, the effect of different light and temperature regimes on the maturation and reproductive success of *H. diversicolor* was evaluated.

## Material and methods

Wild collected individuals of *H. diversicolor* were acclimated under laboratory conditions for two months prior to being transferred to the experimental units in December 2024. A full-factorial experimental design was used, combining two temperature regimes (14°C and a temperature regime of 6-14°C simulating natural temperature variation) with four light regimes (16L:8D, 24L:0D, natural light conditions, accelerated natural light conditions), resulting in seven treatment combinations. Each treatment consisted of four experimental tanks and one reference tank. All groups were fed commercial fish feed twice weekly. In the reference tanks, biweekly sampling was conducted to assess survival, measure individual biomass, document individual condition via photographic analysis, and assess maturation status by extraction of germ cells from a minimum of five individuals per sampling.

After 14 weeks, all remaining were dissected to quantify reproductive output in females through egg counts. At the same time, an exploratory sampling was conducted in the experimental tanks to determine the presence or absence of offspring, with the aim of verifying spawning and reproductive success. Additionally, more comprehensive samplings were scheduled for weeks 20 and 26 to assess later developmental stages and further evaluate reproductive success across treatments.

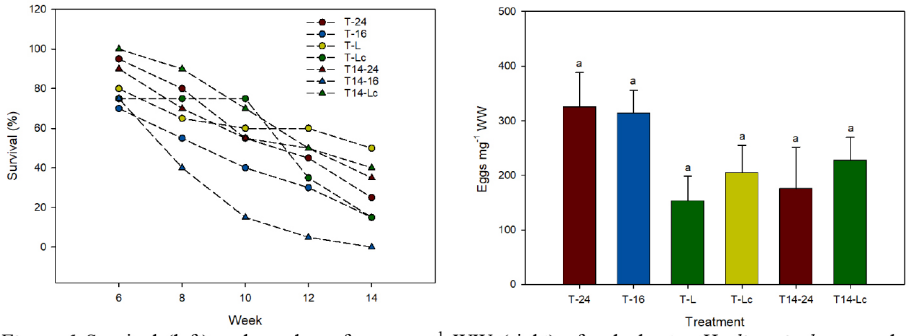


*Figure 1* Survival (left) and number of eggs mg$^{-1}$ WW (right) of polychaetes *H. diversicolor* reared under different light and temperature regimes over a 14 week-period. Same superscripts indicate non-significant differences ($p \geq 0.05$).

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**Preliminary results and discussion**

With the progression of maturation, the survival of *H. diversicolor* decreased across all treatments over time due to its semelparous life history (Hartmann-Schröder et al., 1996). Individuals in the 16L:8D light treatment group, in both natural temperature conditions and at 14°C showed the most rapid decline in survival. Correlating this decline with the onset of reproduction and higher reproductive success in terms of offspring numbers will be crucial for understanding these results, however, this is yet to be evaluated. Variation and light did not affect the final egg $mg^{-1}$ count (one-way ANOVA, $p \geq 0.05$), suggesting no difference in retention of eggs in the coelom.

**Conclusion**

Overall, the light regime appeared to exert a stronger influence on the maturation of *H. diversicolor* than temperature, with more detailed results yet to be assessed. These findings may support the development of large-scale production systems by enabling faster maturation and reproduction.

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# EVOLUTIONARY AND FUNCTIONAL INSIGHTS INTO THE BITTER TASTE SYSTEM OF GILTHEAD SEABREAM (*Sparus aurata*): LINKS TO GUT SENSING AND FEEDING REGULATION

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**Introduction**

Improving feed palatability is a key priority in sustainable aquaculture especially considering the progressive incorporation of alternative ingredients in aquafeeds. These components contain a variety of anti-nutritional factors (ANFs), often associated with a bitter taste, along with adverse effects on fish growth [1]. Bitter tastes are signaled by a GPCR family member, known as type 2 taste receptors (T2Rs) [2]. Despite recent advancements in genomic and functional studies revealing the diversity and roles of T2Rs across a wide range of vertebrates [3], key aspects of T2R biology in aquaculture-relevant species remain underexplored. Understanding bitter functions in this context is especially relevant, as studies in mammals have shown that beyond oral taste, T2Rs can modulate hormones secretion from enteroendocrine (EE) cells, thereby influencing feed intake via the gut-brain axis [4]. In light of these knowledge gaps, this study aimed to characterize the T2R gene repertoire from evolutionary and functional perspectives in the gilthead seabream (*Sparus aurata, sa*), with a particular focus on their potential role as chemosensors for specific ANFs, commonly found in plant-based aquafeeds. Lastly, we search evidence for mRNAs co-expression of *sa*T2R with a subset of anorexigenic EE-peptides, to provide morphological evidences of the potential conserved roles for fish T2Rs in GI-sensing mechanisms regulating appetite and digestion.

**Material and Methods**

Novel fish T2Rs sequences were identified through BLAST/BLAT searches against representative fish genome assemblies (Ensembl, release 111, 2024), using NCBI-annotated teleost T2R protein as queries. A curated dataset (n=125) was then used for phylogenetic reconstructions in MEGA11 [5]. T2R synteny was manually assessed using the Ensembl BLAST MultiTool. *In vitro* responses to known bitter ligands and ANFs were assessed by transiently transfecting the bitter family member *sa*T2R-40 (in pcDNA3) into the stable NFAT-Luc-HEK293 cell line, to drive $Ca^{2+}$-dependent luciferase expression upon receptor activation [6]. Evaluation of ligand responses was based on relative luminescent unit (RLU) mean ± SEM of 8 independent determinations normalized to the mean response of basal levels (% BL). Dual fluorescent *in situ* hybridization (DFISH) was performed using DIG- and fluorescein-labelled riboprobes for *sa*T2R-40, the EEC marker ND1, and the cholecystokinin (CCK) and proglucagon (Pg) genes. Fluorescent signals were developed as previously described [7].

**Results**

BLAST-based gene mining in genome assemblies identified five *sa*T2R paralogs, located on chromosomes 5 (*sa*T2R-40/*sa*T2Rb) and 9 (*sa*T2Rc/d/e), belonging to three distinct phylogenetic clades. Analyses of *sa*T2R syntenic blocks with both closely and distantly related species (including non-teleosts and lobe-finned fish) indicate frequent rearrangements, duplications, and losses across lineages and suggest that *sa*T2R-40/b/e are ancestral gene- paralogs that originated before the tetrapod/ray-finned fish divergence (**Fig.1**), whereas *sa*T2Rc/d most likely arose from whole-genome or segmental duplications at the basis of teleost radiation. Stimulation of *sa*T2R-40-transfected NFAT-Luc-HEK293 cells with the canonical bitter ligand denatonium, as well as with the ANFs genistein and saponin, elicited significant responses that tended to increase in a dose-dependent manner (**Fig.2**). The $Ca^{2+}$ release activator Phorbol 12-myristate 13-acetate (PMA), served as positive control. DFISH experiments provided clear indications for the colocalization of *sa*T2R-40 with the EEC-peptides CCK and Pg (**Fig.3**), supporting the hypothesis that *sa*T2R-sensing in EECs play a central role in the release of anorectic hormones in response to ANFs.

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Fig.1

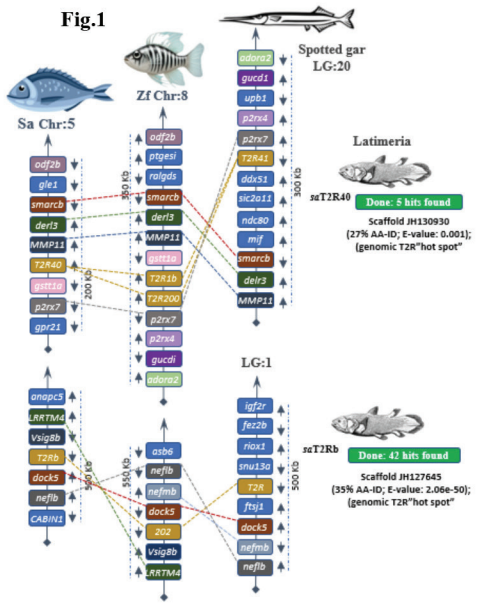



Fig.2

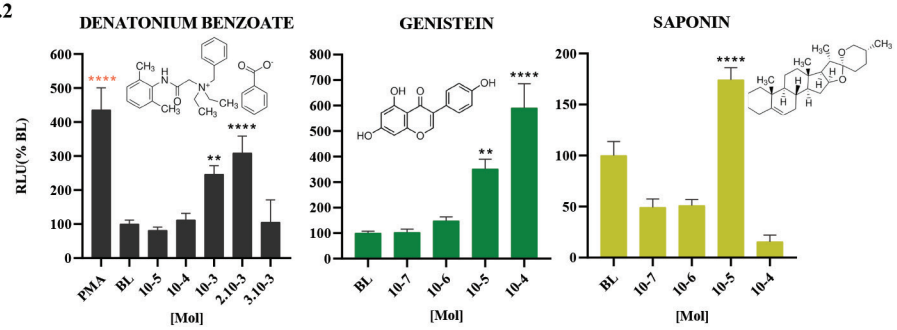
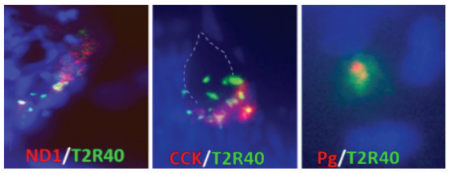



Fig.3




## Conclusions

Globally, these studies provide foundational knowledge that sheds light on the evolutionary and functional aspects of T2R biology, with important implications for applied aquaculture. Beyond their impact on feed palatability, the data support the hypothesis that the detrimental effects of ANFs on fish growth may be mediated, at least in part, by T2R- signalling in the GI tract. This information is instrumental for testing, *in vitro*, potential bitter-masking compounds that could ultimately contribute to the design of novel alternative feed formulations capable of mitigating the release of appetite-suppressing signals from the gut, thereby enhancing feed intake and reducing the environmental impact of feed waste.

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# EXPLORING THE PRODUCTION POTENTIAL OF EMERGING MICROALGAE SPECIES FOR AQUACULTURE APPLICATIONS

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Microalgae play a key role in aquatic ecosystems and are also crucial for the aquaculture sector, particularly during the early life stages in hatcheries (1,2). The use of microalgal biomass supplied by specialized companies, in the form of pastes, concentrates, or powder, has become a practical strategy, offering consistent biomass availability while reducing the risk of microalgae culture crashes that often occur with on-site production (3). It also optimizes space and labour, allowing hatchery staff to focus on the growth and health of their target animals. Therefore, the use of commercial microalgae products is well established in fish hatcheries. However, their adoption in the invertebrate aquaculture sector remains limited, even though most of these organisms rely on algae throughout their entire life cycle. One possible reason is that their specific nutritional requirements differ according to the life stage (larvae, juveniles, adults, and broodstock) and species. In addition, commercial microalgae genera such as *Nannochloropsis* sp., *Tetraselmis* sp. and *Tisochrysis* sp. may not fully meet those needs (3). For this reason, it is essential to investigate other marine microalgae, such as diatoms, as they have been shown to play a crucial role in the nutrition of invertebrates such as bivalves, shrimp, sea cucumber, and sea urchin. Additionally, innovative diets are being investigated as research showed that the inclusion of diatoms as a functional ingredient presented relevant biological benefits, such as immunostimulation (4). One of the first steps in industrial microalgae production is the establishment of species-specific cultivation protocols to enable consistent and scalable biomass production. As part of the Algae Vertical project, production of four diatom species (*Chaetoceros calcitrans, Phaeodactylum tricornutum, Thalassiosira pseudonana* and *Skeletonema costatum*) was performed to characterize their growth performance and biomass productivity. Trials were carried out at an industrial microalgae production facility in southern Portugal under natural outdoor conditions. Most species were cultivated in winter (November-February), except *C. calcitrans,* which was produced during summer (July–August). Cultivations were performed in flat-panel photobioreactors (FP-PBR) along different culture volumes (80–3000L), by transferring the cultures from the smallest systems to the largest, or by splitting the cultures in several reactors of the same volume, to scale up the production. NutriBloom® Plus medium and NutriBloom® Silicates solutions were applied to maintain nitrate and silicate concentrations of 4 mM and 1 mM, respectively, except for *P. tricornuturm*, where only NutriBloom® Plus was used. The growth of the microalgae was monitored through daily optical density measurements. At the end of the production cycle, biomass was harvested by centrifugation, and the yield of the fresh paste was recorded. Following harvesting, biomass was packed and immediately frozen at -20°C for downstream processing (e.g. lyophilization or product formulation). All four diatoms were well adapted to outdoor cultivation in FP-PBR in southern Portugal (Figure 1). *C. calcitrans* reached a productivity of 0.248 $g.L^{-1}.d^{-1}$ within 13 days in 80L, while a slower growth was observed in a 3000L reactor. *T. pseudonana* showed consistent performance across all cultivation volumes, with a maximum productivity also reaching 0.255 $g.L^{-1}.d^{-1}$. *P. tricornutum* exhibited rapid initial growth at 80L, reaching 0.154 $g.L^{-1}.d^{-1}$ productivity, though larger volumes showed a more gradual microalgae growth. *S. costatum* maintained a stable growth at 1000L replicates, with a maximum productivity of 0.244 $g.L^{-1}.d^{-1}$. This work demonstrates the feasibility of producing different diatom species at a pilot scale in an industrial production setting. This represents a step toward the development of new commercial products of microalgae to meet the nutritional requirements of aquatic organisms, particularly for bivalves and other invertebrates. However, to complete the process of microalgae product formulation, further efforts should focus on the biomass harvesting and post-processing steps (e.g. storage and lyophilization). Some of these challenges include damaged *setae* in *C. calcitrans* and colour changes in *S. costatum* and *P. tricornutum* biomass during harvesting or the storage period. These aspects must be addressed to reach a high-quality product for aquaculture applications.

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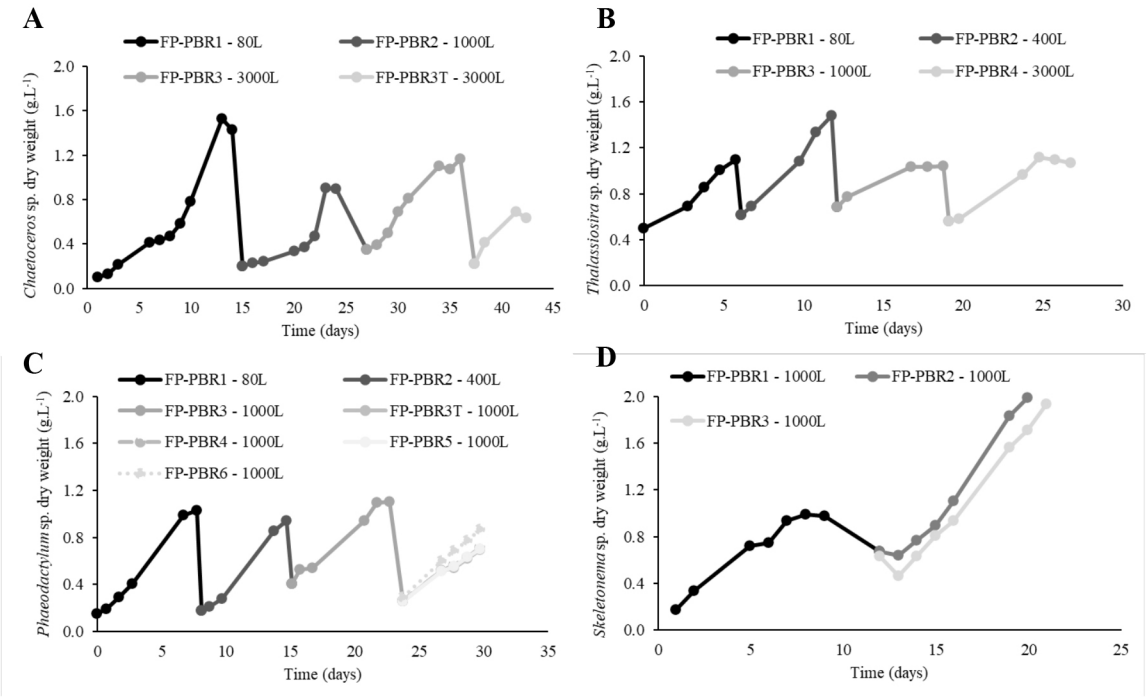


Figure 1 – Microalgae growth of four diatom species in outdoor FP-PBR during scale-up (80L up to 3000L): **A** *Chaetoceros calcitrans* (volumes: 80-3000L); **B** *Thalassiosira pseudonana* (80-3000L); **C** *Phaeodactylum tricornutum* (80-1000L); and **D** *Skeletonema costatum* (1000L). The sudden reduction of biomass concentration indicates an inoculation in a new FP-PBR or scheduled culture transfer to another FP-PBR (T).

**Acknowledgements**
This work was financially supported by "Pacto da Bioeconomia Azul" (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line "Agendas for Business Innovation" through the funding scheme C5 - Capitalization and Business Innovation.

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# EFFECT OF THERMAL TREATMENT ON THE SHELF-LIFE OF MICROALGAE LIQUID CONCENTRATES WITH DIFFERENT BIOMASS DRY WEIGHTS FOR HATCHERY APPLICATIONS

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Microalgae product innovation is essential to improve aquaculture production efficiency, which is crucial for meeting the increasing global demand for fish for human consumption. One of the main challenges lies in reducing the bacterial content in microalgae-based products, as their presence increases the bacterial load and communities in the water of rotifer culture, potentially enhancing the probability of pathologies and outbreaks. This is particularly concerning for marine fish larvae, which are often sensitive to bacterial presence. Commercial liquid concentrates of microalgae tend to carry significant bacterial loads, threatening larvae survival by increasing the microbial population in both rotifer and larvae rearing systems (1–3). The application of thermal treatments in microalgae liquid concentrates has previously shown microbiological benefits (1), but it can induce agglomeration of the product. This creates an additional challenge in the development of microalgae products with reduced bacterial loads, for instance, for recirculating aquaculture systems (RAS) and the green water technique. RAS are very efficient culture systems; however, most microalgae products cannot be applied in these tanks since the presence of microalgae agglomerates clogs the filters, impairing their application in these systems (3). In the green water technique, these agglomerates tend to foster product sedimentation, reducing the water quality and biological efficiency of the fish larvae. Product formulation factors such as salinity, microalgal dry weight and thermal disinfection influence product quality. It is known that different biomass dry weights affect microalgae agglomeration and product sedimentation, which may reduce the water quality and feed availability for the organisms, ultimately impacting their biological performance. This study investigates the effect of low-temperature long-time (LTLT) thermal treatment on *Nannochloropsis* sp. liquid concentrates formulated at different dry weights (8%, 15%, and 18%) under constant salinity, compared to untreated samples (Control). After formulation, the products were frozen and, following one month of storage, thawed and monitored weekly over a four-week refrigerated storage period (secondary shelf life). During this period, product sedimentation in the water column (after 24h) and microbiological parameters (Tryptic Soy Agar - TSA; Plate Count Agar – PCA; and Thiosulfate Citrate Bile Salts Sucrose Agar - TCBS) were assessed. Figure 1 shows the total bacterial content growth in TSA in samples with 8%, 15%, and 18% dry weight under control and LTLT treatment over 0, 1, and 4 weeks.

Bacterial content increased across all liquid concentrates with different biomass dry weights during the storage period. At the end of secondary shelf life (4 weeks refrigerated), control samples consistently showed the highest bacterial loads, particularly at 15% and 18% dry weights. In contrast, LTLT samples showed lower microbial growth at all time points, especially at 15% dry weight, confirming the efficacy of LTLT in reducing microbial growth. These results are in agreement with previous studies, which showed that thermal treatment reduces bacterial load in microalgae concentrates (1). Sedimentation tended to decrease over time; the control samples showed higher pellet heights than LTLT, suggesting that thermal treatment may reduce the aggregation and settling capacity of the biomass, which is beneficial for these product applications in the hatchery. These findings suggest that thermal treatment is a promising strategy to improve the microbiological and physical stability of microalgae concentrates used in aquaculture. From a practical standpoint, this thermal processing approach can extend the secondary shelf life of microalgae-based products due to the reduced bacterial levels and lower product sedimentation, which are key requirements for sensitive aquaculture species and challenging aquaculture systems. Nevertheless, *in vivo* trials in rotifers and fish larvae are needed to validate the biological efficacy of these products under industrial aquaculture conditions. This research supports the development of safer and more stable microalgae-based products that not only meet the organisms' biological needs but also align with operational constraints in modern fish hatchery facilities.

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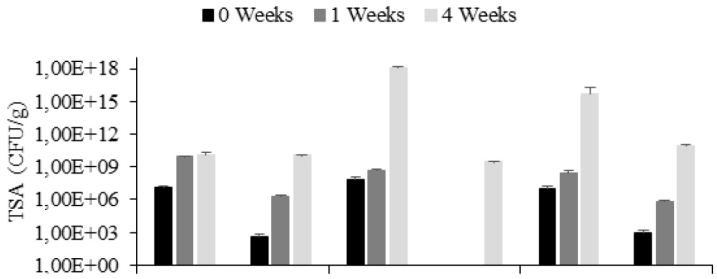


Figure 1 - Quantification of total bacteria on TSA culture medium of *Nannochloropsis* sp. liquid concentrates with different dry weights (8%, 15%, and 18%), subjected to thermal treatment (LTLT) or untreated (Control).

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# BIVALVE AQUACULTURE: EXPLORING ENVIRONMENTAL BENEFITS, GROWTH POTENTIAL AND CHALLENGES IN THE EU

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**Background**
Bivalve aquaculture has been identified as a key sector for the sustainable growth of aquaculture in Europe. However, the reported benefits of bivalve aquaculture for the environment and potential environmental risks have not been well-defined or quantified, and the risk-benefit trade-off remains unclear.

**Methodology and aims**
Firstly, the study aims to detail the positive contributions of bivalve aquaculture in the EU in addressing biodiversity loss, pollution, and climate change (including mitigation efforts), while also considering any possible negative impacts on ecosystems through a systematic literature review. Secondly, a consultation with bivalve aquaculture stakeholders (across several MS) was conducted to develop a SWOT analysis of the current environmental, economic, social, regulatory/institutional, and technological aspects. This analysis seeks to identify opportunities for growth of the bivalve aquaculture by implementing the identified environmental benefits and considering the impacts, focusing on the challenges and opportunities in promoting the benefits of bivalve aquaculture.

The literature review analysis was based on a systematic literature review (SLR) following PSALSAR (Protocol and Reporting result with Search, Appraisal, Synthesis, and Analysis Framework). The project aimed to answer questions regarding the relationship between the bivalve farming sector with key environmental aspects, focusing on the benefits related to biodiversity, pollution and climate change, but also identifying the challenges. Web of Science, Scopus and Google Scholar databases were used. The search was restricted to research items published between 2014 and 2024, using keyword search strings such as “aquaculture AND bivalve”, “farming AND bivalve”, “aquaculture OR farming AND bivalve” and “aquaculture OR farming AND bivalve AND environmental AND benefits” and combinations with “Biodiversity”, “Pollution” and “Climate Change”.

**Preliminary results**
In total, 112 items were selected and after the full text review, 41 research items were finally selected as relevant for this study. These papers were reviewed and summarised as part of the report in order to assess the ecosystem services and related environmental benefits of the bivalve sector. Data collected through the stakeholder consultation was reviewed and critically assessed, and further insights were extracted.

**Conclusion & Outlook**
This study falls under a broader study requested by the European Commission on *Assessing the environmental benefits of aquaculture and challenges and opportunities in promoting those benefits*. The abstract and presentation focuses on the contextual background and project methodological approach, and presentation of results to inform the current state of knowledge of the environmental benefits of bivalve mollusc farming, outlining possible opportunities for development of this type of farming as a low impact type of aquaculture.

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# LOW TROPHIC AQUACULTURE INFRASTRUCTURES AS NATURE BASED SOLUTIONS: ESTABLISHING FRAMEWORKS AND SUSTAINABLE AQUACULTURE PRACTICES

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**Background**

Low trophic aquaculture, including species like bivalves and seaweed, offers a promising opportunity for a sustainable expansion of the global aquaculture while delivering valuable ecosystem goods and services. The EU BIOGEARS project demonstrated that biobased aquaculture gears (ropes) are a more sustainable in aquaculture production than conventional fossil-based options, helping to reduce plastic pollution in suspended bivalve culture through the circular economy. This study aims to evaluate the performance of new biobased ropes with proved mechanical properties, in low trophic mussel and seaweed aquaculture within hybrid blue-grey infrastructures such as offshore longlines and rafts. Integrating biobased aquaculture gears into these infrastructures could enhance their effectiveness as nature-based solutions (NbS). The findings from this study will be used to assess the efficacy of these infrastructures as NbS in TRANSEATION project and to guide the implementation of biobased gears in the aquaculture sector.

**Methodology and aims**

In the TRANSEATION project, two new biobased rope prototypes (T1 and T2) have been developed, enhancing the mechanical characteristics of the best-performing biobased B2 rope prototype in the previous BIOGEARS project. All three biobased prototypes (T1, T2, B2) are based on compounds of commercially available biopolymers and manufactured with industrial processes to be fit-for-purpose, with the required technical and functional properties to be a cost-effici9ent alternative to the conventional fossil-based mussel rope counterpart (GR). Throughout 2024 and early 2025, the setup of a longline and a raft as low trophic aquaculture demonstrators was carried out, which included maintenance and repair activities on the infrastructures. Additionally, several pieces of equipment were installed in the infrastructures, including current meters, pH meters, acoustic sensors, and chlorophyll sensors, aiming to continuously monitor environmental parameters that can impact mussel and seaweed cultivation. Before setting up the cultures, a monitoring plan for the environmental parameters was established, along with the experimental design for the aquaculture trials at sea.

Aiming to evaluate the efficacy of the low trophic aquaculture infrastructures as NbS, the following steps were also undertaken: 1) the development of a System Approach Framework (SAF) into an applicable ecosystem-based management (EBM) Framework that provides a systematic and stepwise guidance for the planning, development, implementation, monitoring and evaluation of hybrid NbS, 2) the establishment of a framework with the identification of indicators to evaluate the benefits and potential trade-offs of these NbS, particularly concerning marine biodiversity and ecosystem services protection and restoration. Finally, in May 2025 the setup of the culture of mussel seeded in biobased rope prototypes (B2, T1, T2) and fossil-based counterparts (GR) was conducted.

**Results**

The preliminary results on the aquaculture performance of the biobased ropes compared to fossil-based counterparts will be presented regarding the evolution of mussel growth and rope mechanical properties. Besides, the adjustment of the established frameworks to the infrastructures will be assessed.

**Results**

Funded by the European Union

European Commission

# FROM WATER BIOREMEDIATION TO SEA URCHIN FEED: *Ulva ohnoi* ENHANCES SUSTAINABILITY IN RECIRCULATING AQUACULTURE SYSTEMS FOR SEA BREAM (*Sparus aurata)* AND SEA URCHIN (*Paracentrotus lividus)* PRODUCTION

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**Introduction**

Recirculating Aquaculture Systems (RAS), particularly when combined with Integrated Multi-Trophic Aquaculture (IMTA), can improve sustainability by enhancing nutrient recycling and fish health (Cesare Marincola et al., 2025)). *Ulva ohnoi* contributes to these systems through bioremediation and nutritional value (Chatzoglou et al., 2020), and its bioactive compounds may help control parasitic infections like monogeneans (Doan et al., 2020). This study assesses a multitrophic RAS integrating *S. aurata* and *U. ohnoi*, focusing on algal productivity, use as feed for *Paracentrotus lividus*, and effects on fish health.

**Material and methods**

Two identical recirculating aquaculture systems (RAS) were used to integrate *U. ohnoi* cultivation with *S. aurata* rearing. In Phase I, macroalgal productivity and its use as exclusive feed for *P. lividus* were assessed. In Phase II, fish were reared in systems with (A) and without (B) macroalgae to evaluate effects on growth and monogenean infection. Each RAS included filtration units, a protein skimmer, two 3 m³ fish tanks, and nine 200 L algae tanks. Algal biomass, fish and urchin growth, and parasite load (assessed via gill scrapes and egg collection on artificial substrates) were monitored. Environmental parameters were recorded. Nutrient excretion was estimated via mass balance, and statistical analyses included GAMs and PCA, with inferential statistics (ANOVA, Dunn's test).

**Results**

In Phase I, *U. ohnoi* exhibited variable biomass gains with significantly higher growth in early autumn (24.4%) and reduced performance during winter (<10%). Biomass production showed no strong correlation with environmental variables (temperature, photoperiod, radiation). Estimated nutrients excretion by fish showed weak correlations with macroalgal productivity. Sea urchins displayed the highest growth during autumn, with declining SGR and gonad index over time, suggesting seasonal effects on feed conversion efficiency. In Phase II, *S. aurata* reared in systems with and without macroalgae showed similar growth performance and nutritional indices. However, monogenean parasite prevalence and egg deposition were consistently lower in the macroalgae-integrated system, particularly at intermediate temperatures (20–21 °C), indicating a potential mitigating effect of *Ulva* on ectoparasite dynamics. Despite a resurgence of infection in late spring, systems with *U. ohnoi* maintained lower parasite loads and egg counts than controls.

**Discussion**

This study demonstrated the viability of integrating *U. ohnoi* cultivation within a multitrophic RAS for *S. aurata*, with macroalgal biomass used to feed *P. lividus*. *U. ohnoi* showed stable productivity across autumn and winter, despite seasonal fluctuations in temperature and radiation. No strong correlation was found between biomass and environmental or nutrient variables, likely due to *Ulva*'s high ecological plasticity and the buffering effect of RAS conditions. Sea urchins fed exclusively with fresh *U. ohnoi* exhibited moderate growth and gonad development, within expected biological ranges, however its sustainability and low cost support its use in integrated systems, and its inclusion in formulated feeds has further demonstrated beneficial effects. For *S. aurata*, macroalgae inclusion had no major effect on growth performance, but ectoparasite prevalence and egg deposition were temporarily reduced in the macroalgae-integrated system. These findings suggest a potential role of *U. ohnoi* in parasite control, though further research is needed to confirm its consistency and mechanisms.

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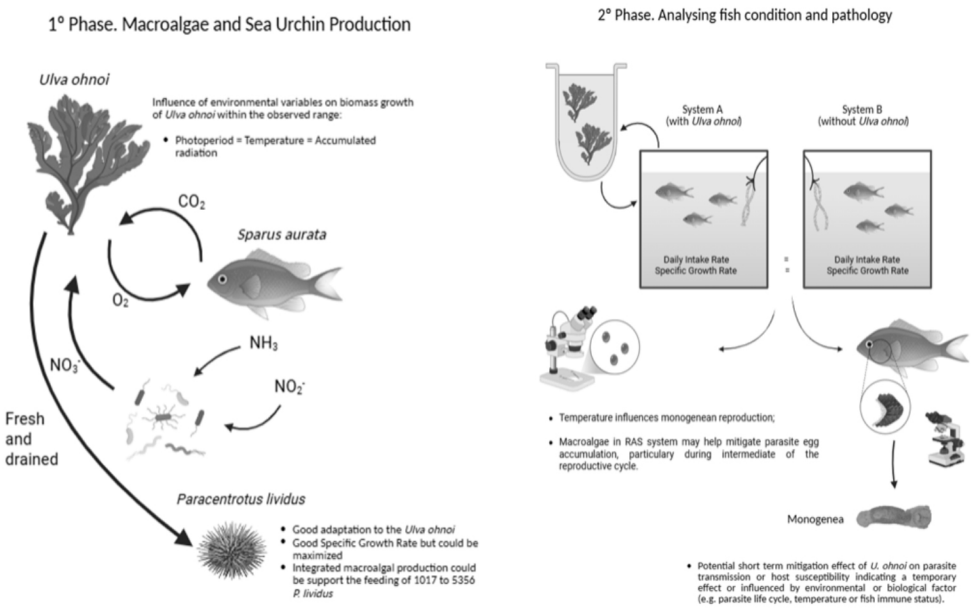


Fig. 1- Experimental design and main results

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# IPMA'S CONTRIBUTION TO ZERO-WASTE AQUACULTURE: VALORIZATION OF SEAWEED AND SEA URCHINS, AND DEVELOPMENT OF FUNCTIONAL PRODUCTS WITHIN AQUAFISH0.0 PROJECT

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**Introduction**

Fisheries and aquaculture are long-established activities in the Atlantic region, representing a key source of economic and social value. These sectors supply a wide range of products for the food, pharmaceutical, and biotechnology industries, yet they also generate large amounts of by-products that are frequently discarded or directed to low-value applications. Increasing awareness of circular economy principles offers an opportunity to transform these underutilized resources into innovative products with higher commercial and societal value. The AQUAFISH0.0 project promotes a sustainable, zero-waste approach by developing high-value products from fisheries and aquaculture by-products, as well as from low-value catches and invasive seaweed species. The consortium involves institutions from Portugal, Spain, France, and Ireland, combining expertise across multiple disciplines. Within this framework, the IPMA is responsible for designing, developing, and characterization of functional products, optimizing sea urchin aquaculture through integrated use of seaweed, and assessing nutritional risks and benefits to provide dietary recommendations. Additionally, IPMA will promote awareness and acceptance of sustainable marine products through education and outreach activities.

**Material and methods**

At the IPMA Aquaculture Station (EPPO), the use of aquaculture by-products in the form of seaweed was tested under two Integrated Multi-Trophic Aquaculture (IMTA) conditions: (A) earthen ponds and (B) a Recirculating Aquaculture System (RAS)-based bioremediation system

A. Earthen Pond trial: A 2250 m² pond was stocked with meagre (*Argyrosomus regius*), gilthead seabream (*Sparus aurata*), and white seabream (*Diplodus sargus*) at a density of 1.3 kg $m^{-3}$. Six floating cages were installed: each inoculated with 2 kg of *Ulva ohnoi* (n=3) or *Gracilaria gracilis* (n=3). Seaweed biomass was harvested every 15 days, and yields were used to feed sea urchins (*Paracentrotus lividus*). The trial lasted 40 days, during which urchin feeding and growth were monitored.

B. RAS trial**:** A recirculating aquaculture system for gilthead seabream (*S. aurata*) was set up, incorporating a bioremediation unit based on *U. ohnoi*. Over 180 days, the system was evaluated in terms of macroalgal biomass production, fish growth performance, and the application of *U. ohnoi* as feed for sea urchins.

C. Future trial with invasive macroalgae**:** The next step will assess the use of the invasive brown seaweed *Rugulopteryx okamurae* as feed for sea urchins (*P. lividus*). This species, with no commercial value and a major negative ecological impact in the Mediterranean and Atlantic, will be tested both as dried biomass and as an ingredient incorporated into formulated inert diets. Control groups will be fed with dried *U. ohnoi* and equivalent inert diets. The trial will evaluate feed acceptance, growth performance, and the sensory quality of gonads.

In parallel, the Aquaculture, Valorization and Bioprospecting Division (DivAV-IPMA) developed a functional food prototype using aquaculture and fisheries by-products as raw material: the red seaweed *G. gracilis*. Two trials were carried out: (1) development of innovative functional food (Sea Nachos"), and (2) evaluation of its nutritional value through physicochemical, biochemical, biological activity (antioxidant and anti-inflammatory properties), sensory, and bioaccessibility study (by using an *in vitro* digestion model that simulates human digestion), also considering culinary treatment.

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**Results**

In the first trial with seaweed cultivated in earthen ponds (A), *Ulva ohnoi* showed excellent adaptation to the culture conditions, with biomass increasing by approximately 126–150% over 15 days. In contrast, *G. gracilis* exhibited poor adaptation, being highly susceptible to contamination by marine fouling organisms, particularly bryozoans. Although an initial biomass increase of 63% was observed during the first 15 days, bryozoan overgrowth subsequently expanded exponentially, ultimately leading to the death of seaweed. In the second trial using the RAS-based bioremediation system (B), *U. ohnoi* again demonstrated strong adaptability to different culture environments, with consistent biomass production. Seasonal differences were observed, with lower yields during the winter months; however, no strong correlation was found between measured environmental variables and production levels. Regarding sea urchin (*P. lividus*) rearing, both trials confirmed high acceptance of macroalgae as feed, supporting adequate growth and normal gonad development in accordance with the species' natural reproductive cycle.

The development of an innovative functional food product demonstrated the potential of aquaculture and fisheries by-products as raw materials for functional applications. The functional food prototype, "Sea Nachos", enriched with the red seaweed *Gracilaria gracilis,* was successfully produced. The "Sea Nachos" were found to be rich in nutrients and bioactive compounds from *G. gracilis*: rich in iodine (10 µg/5 g nacho) and polyphenols (412 mg GAE/100 g dw), as well as, with high biological activity (mainly anti-inflammatory and antioxidant). The *in vitro* digestion assay showed high bioaccessibility of nutrients/bioactive compounds and biological activity, supporting its potential as a sustainable and healthy alternative in human diets. Furthermore, the sensory tests (performed by an international panel) indicated good consumer acceptance.

**Discussion**

The results obtained in earthen pond (A) and RAS (B) IMTA systems demonstrate that both approaches are effective for producing *U. ohnoi* biomass. In contrast, *G. gracilis* proved highly vulnerable to biofouling and therefore unsuitable under the tested conditions. The biomass of *U. ohnoi* showed excellent applicability as a staple feed for sea urchins, supporting a more cost-effective and sustainable aquaculture practice. This highlights the potential of valorizing aquaculture by-products, such as *U. ohnoi*, as a reliable raw material that contributes to circular economy strategies and zero-waste objectives. Regarding the developed functional food ("Sea Nachos"), it was shown that its consumption can make a difference in the promotion of a healthy diet and contribute to a more sustainable society. The "Sea Nachos" may contribute to adequate levels of iodine intake in diet (150 µg/day). Therefore, the incorporation of *G. gracilis* into the formulated nachos has made it possible to develop an affordable, sustainable and healthy product, allowing its consumption to add benefits beyond the usual nutritional ones.

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# APPLICATION OF THE IMTA SYSTEM TO SEA BASS AND FLAT OYSTER FARMS IN THE ADRIATIC BASIN

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**Introduction**

The objectives of the MARINET project (MARIculture NETwork, Interreg Italy-Croatia n. ITHR0200334) include the implementation of innovative technologies to promote diversified and sustainable aquaculture, thereby supporting public health and enhancing regional competitiveness. One of the main goals is to evaluate the applicability and effectiveness of the Integrated Multi-Trophic Aquaculture (IMTA) system when applied to the farming of European sea bass (*Dicentrarchus labrax*) and European flat oyster (*Ostrea edulis*), as developed under Work Package 2.

In recent decades, the inconsistent availability of food sources (particularly phytoplankton) for farmed bivalve molluscs has been one of the notable consequences of the climate crisis. In this context, the IMTA system may offer a promising solution. This study focuses specifically on flat oysters, aiming to support the revival of this historically important bivalve species in the Adriatic Sea. Currently, the Japanese cupped oyster (*Magallana gigas*) has largely replaced the flat oyster in commercial farming. However, *M. gigas* is considered an invasive alien species and is therefore not farmable in Croatia.

This study also aims to evaluate the feasibility of farming flat oysters using wild-caught seed collected in the Adriatic Sea with various trap types. The health status and production performance of the oysters are assessed, along with the effectiveness of different farming methods. Several types of lantern nets are placed at various depths to identify farming conditions that minimize stress for the animals. Finally, the oysters were subjected to bromatological analysis and condition index assessment to evaluate their commercial quality.

**Materials and methods**

Several production sites along the Italian and Croatian coasts were selected, each characterized by different trophic conditions. These were assessed using satellite data on phytoplankton concentrations, silicates, nitrates, and phosphates. The sites included Puglia, Emilia-Romagna, Friuli Venezia Giulia, and the Istrian coast.

At each site, approximately 2000 flat oysters were placed. In IMTA sites, lanterns were positioned both upstream and downstream of the current, with an additional control site located far from the farm. Different types of net and plastic lanterns, along with collectors with varying filtering and accumulation capacities, were used.

**Laboratory Analyses**

Pathogen analysis: cytological, histological and molecular tests.
Health and environmental stress biomarkers: IHC of HSP70, iNOS, Cox-2, Cyp1A and Caspase3.
Chemical analysis: Fatty acid profiles determined using a gas chromatograph (Agilent 7890B) coupled with a flame ionization detector (GC-FID), equipped with a DB-WAX column (Agilent)
Condition index: assessed according to the "Accord interprofessionel sur la denomination et la classification des huîtres creuses"
Phytoplankton analysis: UTERMÖHL technique, UNI EN 15204:2006.

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**Preliminary results**

Analyses are still in progress. In the first phase, several pathogens have been identified, including *Bonamia exitiosa* and *Perkinsus mediterraneus*, with some cases of co-infection. Epibionts such as the sponge *Cliona celata* and the parasitic bivalve *Rocellaria* spp. were also observed. Notably, some individuals showed signs of disseminated neoplasia.

Histological examination of the gonads confirmed active reproductive development even during winter months. In one IMTA farm, an increase in saturated fatty acid levels was observed, particularly in oysters located downstream from the fish farm. Overall oyster mortality has remained low and does not appear to be significantly influenced by the presence of pathogens.

**Discussion**

The detection of *Bonamia exitiosa, Cliona celata*, and *Rocellaria* spp. confirms that the study area is endemic for these pathogens. The presence of *Perkinsus mediterraneus* represents the first report of this parasite in the Adriatic Sea; prior cases had only been documented in the Balearic Islands (2004). The occurrence of disseminated neoplasia in some oysters needs further investigation.

High levels of saturated fatty acids observed downstream of the IMTA system support the hypothesis that flat oysters can utilize organic material derived from fish farm effluents. These preliminary findings suggest a potential benefit of IMTA systems in enhancing the nutritional value of oysters, which will need to be confirmed through stable isotope ratio analysis (C and N).

# PRELIMINARY EVIDENCE OF NURSERY POTENTIAL OF IMTA STRUCTURES FOR THE PURPLE SEA URCHIN *Paracentrotus lividus*

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## Introduction

Integrated Multi-Trophic Aquaculture (IMTA) is a sustainable aquaculture practice that combines species from different trophic levels, typically fish, suspension-feeders, and macroalgae, within a single system to optimize resource use and reduce environmental impacts [1]. IMTA is increasingly promoted worldwide for its potential to curb excess (in)organic matter from high-trophic fish through water bioremediation and nutrient recycling by low-trophic extractive species while diversifying crop production [1, 2].

Artificial substrates used in marine aquaculture, such as ropes and nets, often become heavily colonized by macroalgae and sessile invertebrates such as ascidians, polychaetes, bryozoans and mollusks [3]. These fouling assemblages are considered a serious operational pest due to their damaging effects on farming structures and competition with cultured species, leading to increased maintenance costs for aquaculture sector [4]. However, they can also provide ecological benefits in mariculture environment, such as consuming excess organic matter and mitigating local environmental impacts [5-8].

In a novel multi-species IMTA-converted fish farm in southern Italy, natural fiber ropes were used to cultivate extractive filter feeders from fouling assemblages [9]. The resulting sessile complex fouling community covering the ropes, dominated by the polychaete *Sabella spallanzanii* (Gmelin, 1791), hosted rich associated fauna [7], including juveniles of the purple sea urchin *Paracentrotus lividus* (Lamarck, 1816).

In the Mediterranean, this species has undergone severe population declines due to overfishing and marine heatwaves, especially in the Apulia region (Southern Italy), where a complete fishing ban has been in place since 2023 [10]. However, passive restoration alone may be insufficient and habitat provisioning in anthropogenic environments, such as aquaculture structures, could offer a complementary recovery pathway.

This preliminary study aims to provide the first insights into the nursery potential of the fouling communities highlighting their ecological value and exploring the role of IMTA structures in supporting biodiversity and sea urchin recovery.

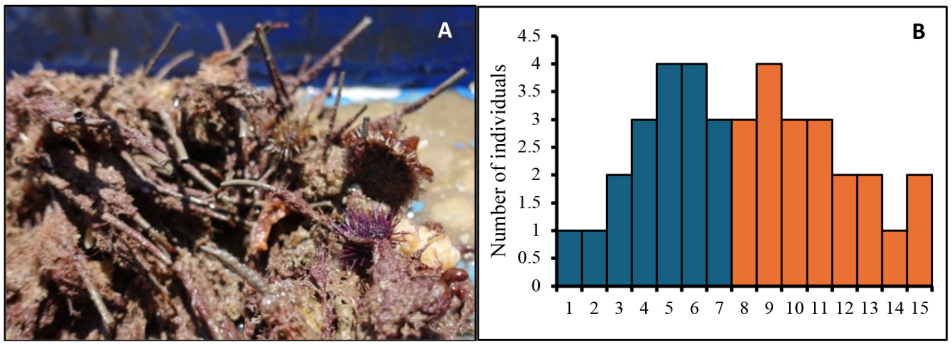


Figure 1. Two specimens of *P. lividus* (purple in the foreground and greenish in the background) found during sampling (A). Size-frequency distribution in two cohorts (blue and orange bars) of individuals of *P. lividus* collected in July 2024. Size classes (mm): 1 = 10-11; 2 = 11-12; 3 = 12-13; 4 = 13-14; 5 = 14-15; 6 = 15-16; 7 = 16-17; 8 = 17-18; 9 = 18-19; 10 = 19-20; 11 = 20-21; 12 = 21-22; 13 = 22-23; 14 = 23-24; 15 = > 24).

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**Materials and methods**

The study area is located in the Mar Grande of Taranto (40°25′56″ N; 17°14′19″ E, Ionian Sea), a semi-enclosed basin connected to the Gulf of Taranto. The investigation was performed in the aquaculture plant "Maricoltura del Mar Grande", hosting the innovative IMTA system. In October 2023, 196 natural fiber ropes (10 m in length) were deployed around fish cages to promote macrofouling growth as IMTA extractive component. In July 2024, six ropes were randomly selected to evaluate the occurrence of juvenile sea urchins. All individuals of *P. lividus* found in the fouling communities were counted and measured in test diameter (without spines) using a digital caliper (resolution 0.1 mm). Based on test diameter, sea urchins were divided into 15 size classes, at 1-mm intervals. Size-frequency distribution was examined to identify cohorts, and mean ± SD were calculated for each. Counts were scaled up to estimate potential abundance across the 196 ropes.

**Results**

A total of 38 individuals of *P.lividus* (Fig. 1A) and 1 of *Arbacia lixula* (Linnaeus, 1758) were recorded, with diameters ranging from 10.0 mm to 24.6 mm. Two size cohorts were identified (Fig. 2A): cohort 1 (n = 18, blue bars), mean diameter = 14.28 ± 1.74 mm; cohort 2 (n = 20, orange bars), mean diameter = 20.38 ± 2.20 mm. The density of the sea urchins was 6.3 ± 5.9 individuals per rope, resulting in a potential production of about 1250 specimens at IMTA farm scale.

**Discussion**

These preliminary observations indicate that fouling communities developing on IMTA ropes may act as nursery habitats for juveniles of the purple sea urchin *P. lividus*, which could be later utilized for repopulation purposes. The detection of two distinct size cohorts suggests multiple recruitment events, a pattern consistent with the extended reproductive period of *P. lividus* in the Ionian Sea [11] and with settlement dynamics observed in other Mediterranean habitats [12]. Based on reported early-stage growth rates of about 2-3 mm/month [13], the individuals of cohort 1 likely settled in early spring 2024, while the larger ones may have originated from recruitment in late autumn-winter 2023-2024.

The species' slow growth and late maturity - typically 3-5 years to reach reproductive size in the Mediterranean Sea [11, 14] - imply that natural population recovery in overfished areas is likely to be a prolonged process, particularly under the additional stress of warming events [10]. While hatchery-based restocking has been attempted [15], these efforts are often constrained by high mortality occurring in both larvae and early juveniles, as well as by the need for cost-effective grow-out systems. In this context, natural recruitment onto aquaculture structures offers a potentially sustainable and low-cost alternative. The fouling assemblages on IMTA ropes, composed of sessile invertebrates, not only mitigated organic matter loading from fish farms [8] but also provide structurally complex habitats resembling marine animal forest [16] that attract a diversity of associated fauna, including species of commercial interest [7]. Although sea urchin abundance on the ropes was highly variable, the IMTA farm has the potential to produce approximately 1,250 individuals of *P. lividus*, which could be reintroduced into natural environments to support population restoration. The scalability of this approach would depend on the total number of ropes deployed in the IMTA system.

Although based on a single sampling event, this study provides preliminary evidence that IMTA ropes can serve as effective nursery habitats for *P. lividus*, offering a cost-effective, nature-based approach that aligns aquaculture production with biodiversity conservation and restoration objectives [17-18]. In their juvenile stage, sea urchins and possibly other organisms find shelter from predators within the forest structured by sabellids and other sessile species, including macroalgae that may also serve as a food source. Long-term, seasonal monitoring will be essential to quantify recruitment patterns, assess in situ growth and survival, and evaluate the feasibility of incorporating IMTA structures into integrated restoration and conservation management strategies.

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# *Cryptocaryon irritans* INFECTION TRIGGERS A HUMORAL IMMUNE RESPONSE AT SYSTEMIC LEVEL IN SHI DRUM (*Umbrina cirrosa*)

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**Introduction**

Species diversification in aquaculture is essential to assure sustainability as well as reducing disease risks. One of the species under evaluation to be introduced in the Mediterranean aquaculture is shi drum (*Umbrina cirrosa*). Their adaptation to captivity, docile handling and their reproduction conditions are excellent characteristics to be considered a good candidate for species diversification (Abellán et al., 2013). However, up to date, very little is known about their susceptibility to pathogens or immunity (Chaves-Pozo et al., 2021). Parasite infections are a great threaten in aquaculture with negative economic impact. Outbreaks of the protozoan ciliate *Cryptocaryon irritans* cause mortalities up to 100% in Mediterranean fish species (Cervera et al., 2022). *C. irritans* complex life cycle difficults its rapid detection and treatment (Li et al., 2021). The objective of this work is to characterize the immune response of shi drum against a severe *C. irritans* infection.

**Material and methods**

In the facilities of Centro Oceanográfico de Murcia (COMU-IEO, CSIC) *C. irritans* outbreaks are quite frequent when temperatures are over 20 ºC. In the case of this study, sudden mortalities, and severe skin injuries appeared in a stock of shi drum. *C. irritans* infection was confirmed by the observation of the trophonts under the binocular loupe. During the 20 days that this infection episode lasted, mortality reached 100%. Samples of serum and head-kidney (HK) were taken in days 1, 2, 3, 4, 5 and 8 from the start of the mortalities (dpi). Homogenates of HK were prepared at a concentration of 1 g of tissue in 1 mL of PBS (pH 7.4). Total levels of proteins were measured by direct UV absorption. Humoral immune response at systemic level was evaluated by analyzing peroxidase, antiprotease and bactericidal activities in serum and HK homogenates following previously described protocols (Cervera et al., 2022).

**Results**

Peroxidase activity increased along the time in both HK and serum reaching the highest level at 8 dpi. Antiprotease activity was significantly increased in the HK at 1 dpi but in the serum did at 5 dpi, returning then to similar levels as the control. In both cases, bactericidal activity decreased along the time becoming undetectable from day 3 onwards in serum, while in HK a progressive decrease was observed from day 4 onwards.

**Discussion and conclusions**

Systemic humoral response of shi drum was modulated by *C. irritans* infection. The fact that only peroxidase activity increased along the infection point to an inefficient way to control the disease. In fact, the transitory increase of antiprotease activity, key to neutralize the parasite proteases (Siqueira-Neto et al., 2018), occured at the same time as the blockage of the bactericidal activity. These data point to certain immunosuppression triggered by the parasite to evade the immune response as described for other parasites (Holzer et al., 2021).

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# MOLECULAR CHARACTERIZATION OF BETA-DEFENSIN TRANSCRIPTS IN THE EUROPEAN SEA BASS TESTIS

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**Introduction**

Antimicrobial peptides (AMPs) are short aminoacidic peptides widespread in nature. AMPs can modulate the host immune response and directly kill pathogens such as viruses, bacteria, or parasites (Katzenback, 2015). AMPs are a very diverse group, which is divided into families. In this project, we focused on beta-defensins (Bdef), a family characterized by the presence of conserved cysteines and disulfide bonds (Das et al., 2022). The presence of Bdef in reproductive tracts has been reported, especially the males, in which Bdef plays its role in reproduction and immune-endocrine regulation (Jing et al., 2010). In teleost fish, several pathogens such as betanodavirus (NNV) can colonize the gonad causing latent infection, which can be transmitted to their progeny (Valero et al., 2018). In this case, NNV control in the gonad results key to overcome potential outbreaks in fish farms. Basing on the available literature, the objective of this work is to deepen in the molecular characterization of the two *bdef* transcripts in European sea bass (*Dicentrarchus labrax*), *bdef1* and *bdef2*, upon diverse conditions in testes.

**Material and methods**

Testes and ovaries from a broodstock of European sea bass were collected. Testes of healthy specimens of European sea bass were sampled monthly to study Bdefs gene expression during the male reproductive cycle (Valero et al., 2015a). Also, males were injected with culture medium alone or containing $10^6$ $TCID_{50}$ of RGNNV/fish. Testes were removed after 1-, 7- and 15-days post-infection (dpi) (Valero et al., 2015b). All samples were processed for gene expression analysis using qPCR.

**Results**

The expression levels of *bdef1* were similar in testis and ovary while *bdef2* expression was higher in testis. Besides, the transcriptional levels of *bdef2* were higher than *bdef1* in testis. During the male reproductive cycle, the highest expression of *bdef1* occurred during spermatogenesis stage while *bdef2* was more expressed in spawning stage. During NNV *in vivo* infection *bdef1* was up-regulated at 7 dpi while *bdef2* remained unaltered.

**Discussion and conclusions**

The transcription of *bdef* in European sea bass gonads has been reported for the first time. As for other species Bdef, *bdef2* seems to be preferentially expressed in the male reproductive tract (Jing et al., 2010), pointing to their role in sperm preservation. Considering this, we have focused on the expression of *bdef* in males. Along the male reproductive cycle, *bdef1* and *bdef2* resulted differently modulated suggesting different functionalities for each gene. The pattern of expression of *bdef1* in gonad upon NNV infection is similar to another AMPs (Valero et al., 2015). Our results point to *bdef1* as an antiviral molecule in the testes. Further studies are mandatory to clarify the differences in regulation of both transcripts.

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# COMPARING EPIDEMICS AND CONTROL MEASURES FOR SALMONID DISEASES USING NETWORK MODELLING

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**Introduction**

Infectious diseases pose a major threat to aquaculture sustainability. Once introduced, aquatic diseases can propagate rapidly through highly connected aquaculture sites and cause substantial production losses due to increased mortalities. Surveillance and control measures such as contact tracing, culling and fallowing are vital for preventing this spread and mitigating impacts on both the industry and the environment. However, the epidemiological and economic impact of these activities may differ depending on the characteristics of the controlled disease. By improving our understanding of specific disease dynamics within aquaculture networks, control strategies can be tailored to be the most effective and impactful, whilst minimising the economic burden to the industry and the competent authority.

England & Wales are free from infectious hematopoietic necrosis virus (IHNv) and viral haemorrhagic septicaemia virus (VHSv), two serious salmonid diseases listed by the World Organisation for Animal Health (WOAH) and nationally controlled under the Aquatic Animal Health (England & Wales) Regulations (2009). Using a network modelling framework, this study aims to compare the complex interplay of epidemics and control measures between the two diseases and contribute evidence to inform tailored disease preparedness.

**Materials and Methods**

The modelling framework, AquaNet-Mod, was adapted and applied to IHNv and VHSv outbreaks in salmonid aquaculture in England & Wales (Guilder et al. 2023). AquaNet-Mod simulates disease transmission within the network via river connections and live fish movements, integrating data collected by the competent authority through statutory monitoring.

Here, an extensive literature review and expert elicitation was conducted for parametrising specific disease characteristics in the model, such as life cycle stages and seasonal changes. Subsequently, a comparative analysis was conducted to compare the epidemic dynamics between the two diseases and evaluate the effectiveness of various control measures from an epidemiological and economic perspective. Finally, disease characteristics that drive differences in transmission dynamics and efficiency of control measures were identified using sensitivity analysis.

**Results**

This study found significant differences in disease dynamics throughout the England & Wales aquaculture network, with IHNv epidemics resulting in larger and more costly epidemics than VHSv. For instance, the average overall cost of a IHNv epidemic was 26% higher than an VHSv epidemic under the current control measures in England & Wales. Control measures were, however, shown to similarly prevent the spread and impact of both diseases, notably with a significant decrease in the size, duration and cost of the epidemics. Subsequently, the importance of specific disease control measures, particularly contact tracing, catchment controls and fallowing period were shown. Key disease characteristics, such as seasonality and virus decay rate in sediment, were identified as primary drivers of these differences in transmission and impact.

**Discussion**

This study facilitates key comparisons between the dynamics of two serious salmonid diseases, aiding informed decision-making with respect to tailored disease control management. The results of this study confirm the vital role disease control strategies play in preventing larger, more costly epidemics, regardless of the disease. Furthermore, it shows the value of mathematical modelling frameworks in better understanding complex interactions between epidemiological and economic impacts of epidemics and control measures, providing evidence to justify the application of effective balanced control strategies, informing resource allocation and enhancing disease preparedness.

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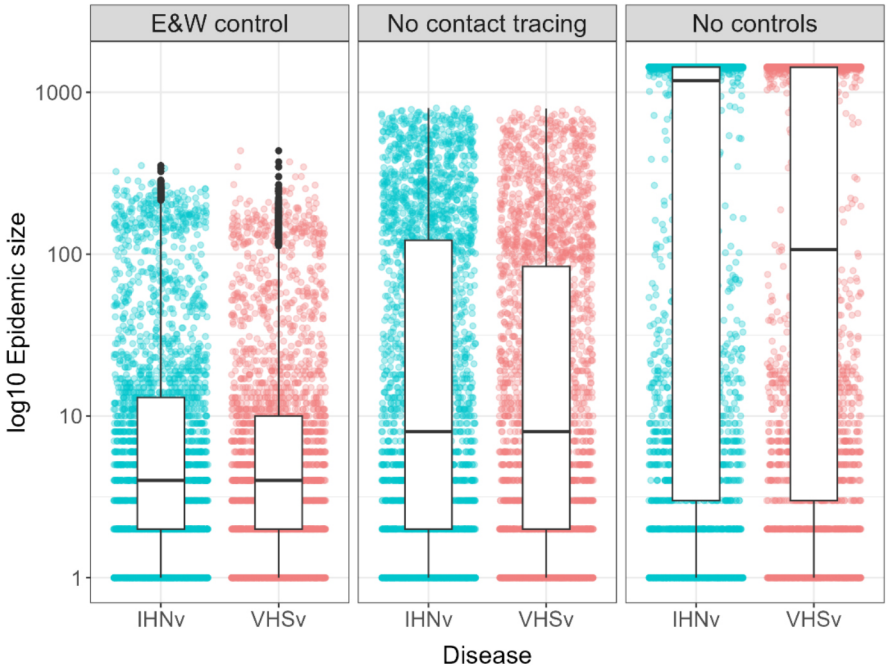


**Figure 1.** Box plot displaying the epidemic size defined as the number of sites infected throughout the duration of the epidemic for both IHNv (left bar, blue) and VHSv (right bar, pink) from simulations conducted in the England &Wales salmonid network under current control measures in (left), contact tracing removed (middle), and all controls removed (right). This data is derived from 3,000 simulations, each lasting 10 years or until the infection ceases for each scenario. The epidemic size is represented using a log-10 scale.

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# ROBUST lOCALIZATION AND mAPPING FOR uuvS OPERATING IN INDUSTRIAL SCALE FISH FARMS

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## Introduction

The aquaculture industry has seen rapid growth over the last decades. This growth, however, presents new challenges in terms of ensuring efficient, safe, and sustainable operations [1]. Fish farming often involve a significant amount of manual labor, which can be physically demanding and dangerous. Tasks such as net inspection, maintenance, and repairs expose workers to hazardous underwater conditions, including rough seas, low visibility, and the presence of potentially harmful marine life. Addressing some of these problems, the interest in robotic systems for aquaculture has also grown significantly in recent years [1]. However, in order to have robust systems operating in dynamic environments we need to increase the level of autonomy of underwater vehicles operating in fish farms. This paper presents a general framework that integrates visual and acoustic sensor data to enhance localization and mapping in complex, highly dynamic underwater environments, with a particular focus on fish farming.

This work was financed by the Research Council of Norway through the project: CHANGE [2] and ResiFarm [3].

## Materials and methods

Fig. 1 provides a brief overview of the proposed underwater localization and mapping framework, which allows UUVs to operate in dynamic underwater environments [4]. The proposed framework investigates vision-based methods to: 1) obtain the relative 3D pose of an UUV from a flexible and deformable structure to facilitate control strategies for autonomous net inspection operations in fish farms, 2) construct the depth map from mono-vision data that can be crucial for both collision-free autonomous operations with UUVs in dynamic environments and for obtaining the real-time map of the inspected area to identify irregularities such as holes, or biofouling in net pens, 3) estimate the global pose of an UUV within the net pen utilizing the available sensor measurements and relative poses of the robot, and 4) create a detailed 3D map of the net-pen environment utilizing data obtained from an industrial scale fish farms (Fig. 1).

## Results

This section presents evaluations across four key areas: relative poses, depth image predictions, global pose estimates, and mapping [4]. Field trials have been conducted in SINTEF ACE facilities to obtain the datasets for validation of the method. Fig. 2 shows the net relative distances estimated where the distance measurements from the DVL and the forward-facing ping echo sounder are compared with distance estimates obtained from the modified FFT-based method (Method 1), the TRU-depth (Method 2) and the Radar Meets Vision (Method 3), while Fig. 3 and Fig. 4 show the depth predictions and obtained map.

## Conclusion and future work

This paper presents a vision-based framework for underwater localization and mapping, using a large dataset from industrial fish farms. The approach employs FFT-based priors to support TRU-Depth and Radar Meets Vision methods, enabling depth prediction from monocular images for 3D mapping. It also proposes techniques for estimating both net-relative and global poses of UUVs. Results show that combining depth prediction, wavemap methods, and pose estimation produces accurate volumetric maps, demonstrating real-world potential in underwater environments. Future work could integrate priors based on fish or other structures to enhance 3D scene reconstruction.

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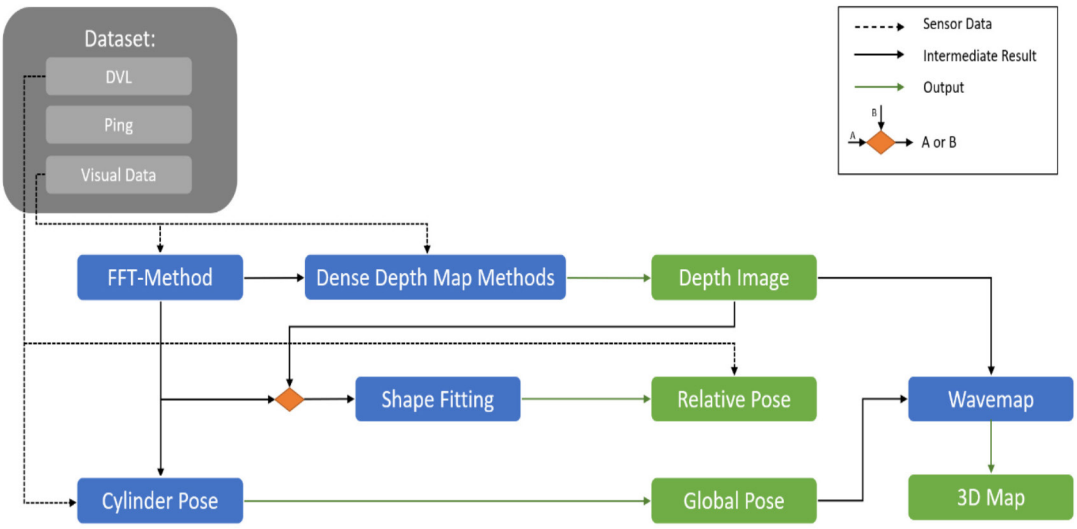



*Figure 1 Overview of the general framework for localization and mapping for UUVs operating in dynamically changing environments*

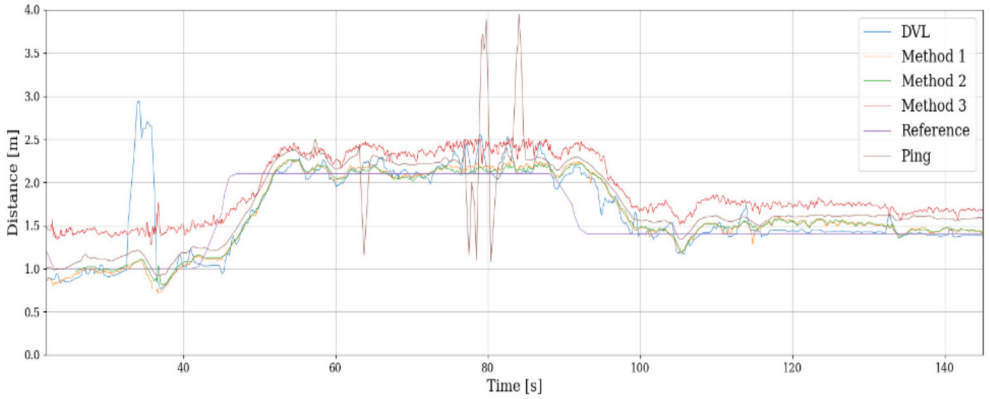
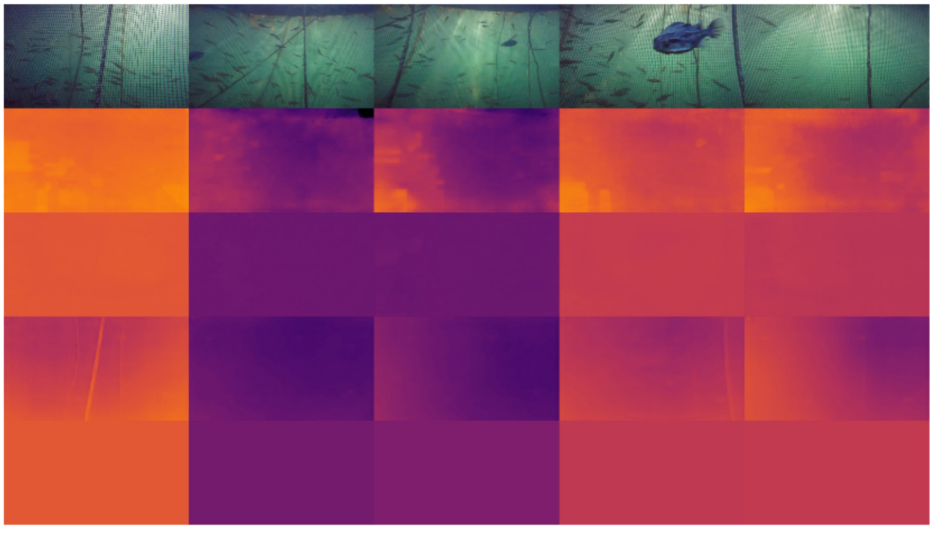



*Figure 2 Net relative distance results — DVL: Estimation from DVL plane approximation; Method 1: Modified FFT; Method 2: TRU-depth utilizing all the obtained FFT priors; Method 3: Radar Meets Vision utilizing up to 5 FFT priors; Reference: Desired input to UUV to follow constant distance from the net pen; Ping: Data from Ping sensor.*



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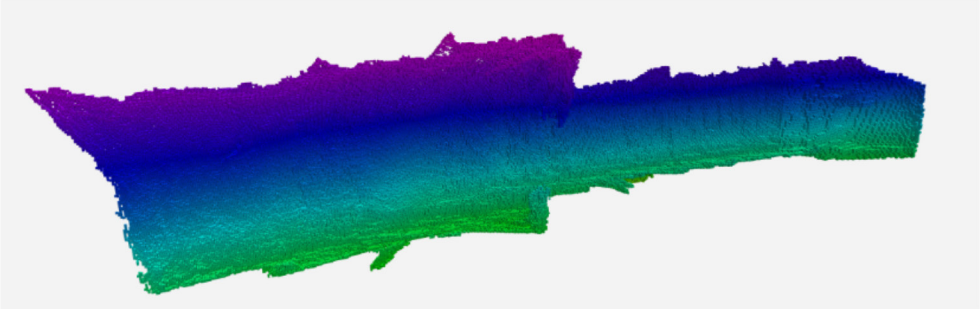

*Figure 4 Volumetric maps generated by the wavemap method.*

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# HIGH LIPID CONTENT DIETS ENHANCE GROWTH AND IMPROVE ELEMENTAL STOICHIOMETRIC ADJUSTMENTS IN *Ruditapes decussatus*

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## Introduction

Two variables are crucial in the intensive production of marine bivalves: the biochemical composition (BC) of food supplied together with the occurrence of large inter-individual differences in growth performance of endogenous origin. Quantitative knowledge of the effects of these variables and their interactions is of great interest for hatchery management, providing both useful information for the formulation of optimal diets and criteria for the eventual selection of high performance lines. Regarding BC, the effects of variable proportions of lipid to carbohydrate in the diet has been extensively studied (mainly in clams) in the search for non-phytoplanktonic supplements that can reduce phytoplankton production costs in bivalve rearing (Laing, 1987; Albentosa et al., 2002; Mamat & Alfaro, 2014). The present study investigates the effects of mixed diets with varying lipid/carbohydrate ratios on a) the growth and physiological performance and b) the elemental (C, N) composition and stoichiometric balances in fast- and slow-growing phenotypes of juvenile carpet shell clams (*Ruditapes decussatus*), focusing on homeostatic nutrient regulation, stoichiometric adjustments, and biochemical composition of body tissues.

## Materials and methods

Juvenile clams from the same cohort were segregated into fast (F) and slow (S) growing groups. They were subjected to a controlled diet comprising varying lipid/carbohydrate ratios, with constant C:N ratios. The composition of the diet differed according to the proportions of the microalga *Rhodomonas lens* and the yeast *Saccharomyces cerevisiae* (D1: 80% - 20%; D2: 50% - 50%; and D3: 20% 80%, respectively). Physiological parameters such as clearance rate, absorption efficiency, ammonium excretion rate and metabolic rate were measured to determine the scope for growth (SFG). These parameters were then utilised to calculate elemental balances (C and N) by means of analysis of their C and N contents in food and faeces. Finally, the clams were freeze-dried in order to ascertain their biochemical composition by means of conventional colorimetric procedures (see Arranz et al., 2021).

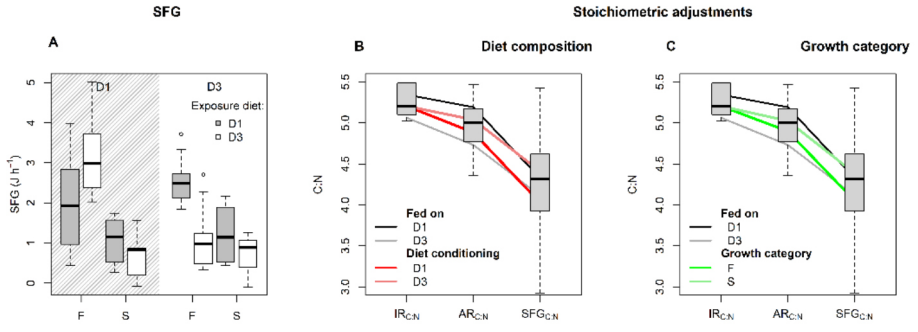


Figure 1. *A)* SFG (standardized values) of F and S clams exposed to diets D1 (gray boxes) and D3 (white boxes), and conditioned to diets D1 (shaded areas) and D3 (white areas). *B)* and *C)* Stoichiometric adjustments represented by C:N ratios for IR, AR and SFG. The lines represent the trajectory of each group combination, where the effects of feeding (exposure diet) on either D1 (black) or D3 (grey) are combined with the effects of conditioning (*1B*, red lines) and growth category (*1C*, green lines).

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**Results**

Dietary effects: Lipid-rich diets significantly enhanced SFG (Figure 1A) and elemental balances in juvenile clams. Clams conditioned to lipid-rich diets exhibited higher energy balances compared to those fed carbohydrate-rich diets. The biochemical composition of body tissues reflected the dietary content, indicating limited ability for homeostatic compensation. However, F clams showed a higher degree of tissues homeostasis.

Phenotypic differences: Fast-growing clams (F) showed greater phenotypic plasticity compared to slow-growing clams (S). This plasticity allowed fast-growing clams to better manage nutritional limitations imposed by different diets. For instance, fast-growing clams increased their clearance rates by 25% when fed carbohydrate-rich diets, compensating for the lower energy content (Figure 1A). In contrast, slow-growing clams exhibited only a 10% increase in clearance rates under the same conditions, indicating a lower capacity for compensatory feeding.

Stoichiometric Adjustments: Stoichiometric adjustments (Figure 1B and 1C) were primarily post-absorptive, with significant differences observed between diets and growth phenotypes. Fast-growing clams maintained a more stable C:N ratio (around 4.0) in their tissues across different diets, suggesting efficient nutrient regulation, while slow-growing clams exhibited a wider range of C:N ratios (3.8 to 4.5).

**Discussion and conclusion**

Conditioning to a high lipid content diet enhanced physiological compensations, allowing juvenile clams to face acute nutritional deficits. In contrast, the low lipid content diet reduced digestibility and impaired lipid balances. Lipid-rich diets significantly improved growth performance, especially in fast-growing phenotypes, highlighting the endogenous origin of growth differences. Fast growers achieved better physiological status under high-quality diets, enabling effective compensation for acute changes. Slow growers showed higher nitrogen release, indicating less efficient protein turnover. Stoichiometric regulation involved changes in absorption patterns and postabsorptive regulation.

The enhanced growth rates and stoichiometric balances observed in clams fed lipid-rich diets highlight the importance of dietary lipid content for optimizing growth and physiological efficiency in bivalve aquaculture. Given the greater phenotypic plasticity and together with their higher degree of tissues homeostasis, the selection of fast-growing lines allows them to be more resilient to nutritional variations. However, replacing more than 50% of microalgal diets with inert diets can compromise growth and disrupt lipid and protein balances.

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# HOW MANY MARKERS DO I NEED? OPTIMAL MARKER DENSITY AND GENOTYPING TOOL CHOICE FOR LONG-TERM GENETIC SUCCESS IN AQUACULTURE BREEDING PROGRAMS

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Achieving sustained genetic progress in aquaculture breeding depends on selecting genotyping tools with the optimal number of markers for a given breeding strategy. This presentation explores key factors influencing marker density requirements, including the number of traits, genome size, linkage disequilibrium (LD) breakdown, recombination rate, genotype-by-environment interactions (GxE), and the need for precise selection over multiple generations.

Multi-trait breeding programs require higher marker densities to capture genetic variation across traits such as growth, disease resistance, quality, and reproduction. Genome size also impacts marker density needs, influencing genome-wide coverage. LD breakdown and recombination rates affect marker effectiveness, requiring strategic placement to ensure critical trait regions are adequately covered. Balancing marker density with LD decay and recombination events is essential for long-term breeding program success.

This presentation highlights the importance of an adaptive approach to marker density planning, ensuring genotyping tools align with breeding program goals. By optimizing marker selection, aquaculture breeding programs can enhance genetic improvement efficiency, maximize selection accuracy, and sustain progress over generations.

# FROM WASTE TO FEED: TURNING FOREST BY-PRODUCTS INTO SUSTAINABLE INGREDIENTS FOR FISH FEED

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**Introduction:**
Aquaculture's rapid growth is driving demand for sustainable protein and fat sources, yet reliance on wild-caught fish and crops contributes to overfishing, land-use pressure, and environmental impacts. Lignocellulosic sidestreams, abundant by-products from forestry, agriculture, and bio-based industries, offer a promising sustainable alternative. In the Nordic region, the forest-based industry produces large volumes of lignocellulosic sidestreams such as lignocellulosic hydrolysates. This study evaluates selected sidestreams from mills in Nordic countries as fermentation feedstocks for microorganisms like bacteria, fungi and yeast, assessing their potential to produce lipids and proteins for sustainable aquaculture feed.

**Main results**
Lignocellulosic sidestreams from pulp and paper industries in Sweden and Norway including lignocellulosic hydrolysates from hardwood and softwood, were collected, homogenized, freeze-dried, and stored before use. Microorganisms like oleaginous yeast strains and fungi were cultivated in bioreactors containing 10 g $L^{-1}$ of each material, with fermentation conducted for six days under controlled and optimized conditions. Comprehensive analyses were performed before and after fermentation, including composition (cellulose, hemicellulose, lignin), CHN content, sugars, phenolic compounds, inhibitors, metals, lipid yields, and fatty acid profiles. Lipids were extracted using the Folch method, converted to FAMEs, and analyzed by GC, while sugars and inhibitors were quantified via HPAEC-PAD and HPLC, phenolics by LC–MS/MS.

**Results:**
This study demonstrates that side-streams from the pulp and paper industry can serve as fermentation feedstocks for microorganisms, enabling the production of high-value food and feed ingredients. Certain yeast strains were able to consume available sugars and showed the ability to metabolize phenolic compounds, suggesting that lignin-derived carbon could be converted into lipids and proteins through optimized bioprocessing. We obtained promising results in the production of omega-3, protein, and astaxanthin.

**Acknowledgements:**
The financial support by the projects, CirkFerm (InterReg) and Circular Biomass 3.0, is greatly appreciated.

# AGGLOMERATION IN NORWEGIAN SALMON AQUACULTURE REVISITED: THE INDUSTRY HAS MATURED

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The importance of industry clusters may vary over time. The benefits of being located in an industry cluster is normally largest in innovation intensive periods which often occurs in the initial phase as the inception of an industry. This can be important for industry location and regional policies, as companies located outside of an industry cluster is less likely to succeed when agglomeration externalities are important, while there may be no disadvantages associated with staying outside of the cluster for a more mature industry.

In this paper we investigate the presence of agglomeration externalities for the Norwegian salmon industry using a translog cost function. This is an industry where there is extensive evidence of industry clusters, but with highly dated data. There is increasing evidence that the industry is maturing and productivity growth is slowing down, and larger companies with internal knowledge networks is becoming increasingly important.

We find that there is a structural break in the estimated parameters between 2015 and 2016. As in earlier studies, we find evidence of agglomeration externalities in the first part of our data set. However, these disappears after 2015, changing the considerations that should be made when locating farms as well as the costs associated with awarding licenses based on regional policy considerations.

# DEVELOPMENT OF A CATIONIC LIPID-BASED NANOVACCINE INCORPORATING RECOMBINANT ISKNV MAJOR CAPSID PROTEIN FOR ASIAN SEABASS

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## Introduction

Infectious spleen and kidney necrosis virus (ISKNV) poses a significant threat to Asian seabass (*Lates calcarifer*) aquaculture in Thailand, causing high mortality rates and economic losses. Traditional vaccination methods, such as intramuscular injections, are often impractical for large-scale operations due to the stress they impose on fish and logistical challenges. Consequently, alternative strategies like nanotechnology-based immersion vaccines are being explored to enhance vaccine efficacy and feasibility in aquaculture settings.

## Materials and Methods

ISKNV infection in diseased Asian seabass was confirmed via PCR assays. The major capsid protein (MCP) gene was amplified, cloned, and expressed in *E. coli* to produce recombinant MCP (rMCP), which was purified and structurally validated using SDS-PAGE, immunoblotting, and transmission electron microscopy (TEM). Cationic lipid nanoparticles were synthesized to encapsulate the rMCP at various ratios, and their physicochemical properties - including particle size, zeta potential, and polydispersity index (PDI) - were characterized to determine the optimal formulation. Immersion vaccination trials were conducted on juvenile Asian seabass, and immunological responses were assessed by measuring IgM levels and the expression of immune-related genes using quantitative real-time PCR (RT-qPCR).

## Results

The 1:2 rMCP-to-nanoparticle ratio showed optimal traits: <200 nm size (Fig. 1), positive zeta potential, and lowest PDI.

## Discussion

While injection vaccination remains prevalent, it is labor-intensive and unsuitable for small fish (Priya & Kappalli, 2022). Oral vaccines face challenges like antigen degradation in the gastrointestinal tract (Jin et al., 2021). Immersion vaccination emerges as a practical alternative, effectively stimulating systemic and mucosal immunity with less labor (Bøgwald & Dalmo, 2019). This study developed a CTAB-based nanoparticle immersion vaccine using recombinant ISKNV MCP, enhancing immune responses in juvenile Asian seabass. A 10 mg/L concentration was selected based on prior studies balancing efficacy and cost (Zhao et al., 2019). Challenges included the absence of specific anti-MCP IgM antibodies, limiting quantitative ELISA assessments, and difficulties in amplifying T cell-related genes. Future research should focus on the ISKNV challenge following treatment and explore broader applications across various fish species.

## Conclusion

The recombinant ISKNV MCP nanovaccine, administered via immersion, holds promise for ISKNV prevention in aquaculture.

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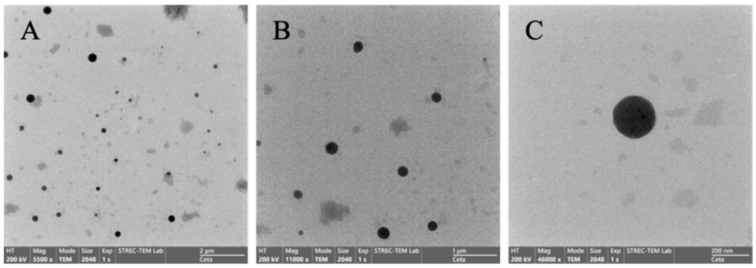


**Fig. 1** The rMCP of the ISKNV, combined with CTAB nanoparticles, was stained with 1% PTA and visualized using a TEM at 5,500 × (A), 11,000 × (B), and 46,000 × (C).

Fish treated with the rMCP nanovaccine showed significantly higher IgM levels (Fig. 2) and upregulated immune-related gene expression was observed at 14 and 24 days post-treatment.

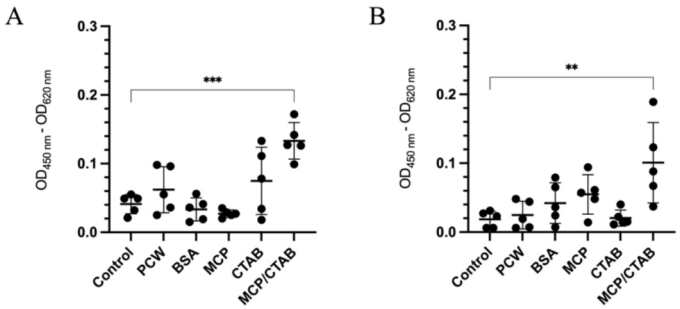


**Fig. 2** The level seabass at 14 days (A) and 24 days (B) post-treatment. Data are presented as mean ± standard deviation, with statistical significance indicated by ** ($p < 0.01$) and *** ($p < 0.005$).

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# EVALUATION OF CHITOSAN BASED SOLUTIONS FOR FISH FILLETS PRESERVATION

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## Introduction

Due to the extended use of plastic and the associated environmental challenges, the European Union has introduced legislation to limit plastic production and waste accumulation. The European Parliament and Council (2024) support the use of eco-friendly packaging materials. By 2030, all plastic used in packaging must be fully recyclable. The use of biobased plastics from renewable resources aligns with these goals. Chitosan forms biodegradable, bioactive and biocompatible coatings. Its antimicrobial activity is attributed to cell membrane disruption, interactions with microbial gene expression, nutrient chelation, and the generation of reactive oxygen species (Khubiev et al. 2023). Global aquaculture reached 130.9 million tons in 2022 (FAO, 2023), but fish and seafood products are highly perishable due to their nutrient value, high moisture content, near-neutral pH, and high levels of unsaturated lipids and non-protein nitrogenous compounds (Kontominas et al. 2021). As a result, the selection of appropriate and environmentally friendly packaging materials is crucial to maintaining the quality of fish (Tsironi and Taoukis 2018). In the present study, active chitosan-based coating solutions were applied to fresh gilthead seabream fillets (*Sparus aurata*) to assess their potential for extending shelf life.

## Materials and methods

Chitosan-based solutions was produced by dissolving 1% w/v chitosan in 0.5% v/v acetic acid aqueous solution at 35°C. A 0.2% v/v organic citrus extract (coded as flavomix) was added as an active compound. The solutions were applied as coating materials on fresh gilthead seabream (*Sparus aurata*) fillets by immersing the fillets in the solution for 15 min. The samples were then dried at 2°C for at least 2 h. After drying, the samples were placed at sterilized Petri dishes and stored isothermally at 2°C. Three groups of samples were produced: uncoated samples (control), samples coated with 1% w/v chitosan (coating 1) and samples coated with 1% w/v chitosan enhanced with 0.2% w/v flavomix (coating 2). The three groups were subjected to shelf life evaluation by determining microbial growth, specifically Total Viable Count (TVC) and *Pseudomonas* spp. During the storage period, the optical appearance of the samples were evaluated using the CIELab system.

## Results and Discussion

Figure 1 presents the growth curves of TVC and *Pseudomonas* spp. fitted to the Baranyi Growth Model. The curves did not exhibit a lag phase. The initial load for TVC was 4.37, with no statistically significant differences between the three tested groups. Similar values have been reported by Koutsoumanis et al. (2002) and Spanou et al. (2024) as the initial microbial load of Mediterranean species. Control samples exhibited the highest growth rates for TVC and *Pseudomonas* spp. ($1.01\pm0.16$ $d^{-1}$ and $1.01\pm0.13$ $d^{-1}$, respectively). The growth rate of samples treated with coating 1 was $0.81\pm0.04$ $d^{-1}$ for TVC and $0.86\pm0.01$ $d^{-1}$ for *Pseudomonas* spp. Samples with coating 2 had a growth rate $0.44\pm0.02$ $d^{-1}$ for TVC and $0.60\pm0.02$ $d^{-1}$ for *Pseudomonas* spp. The maximum load of TVC ranged between 9.85-9.97 logCFU/g and 8.99-10.81 logCFU/g for *Pseudomonas* spp. According to Alexi et al. (2022) the limit of fish acceptability can be considered as 7 logCFU/g for TVC. Based on this limit, the shelf life of control samples was 2 days. The shelf life of samples treated with coating 1 was 4 days and the shelf life of samples treated with coating 2 was 6 days.

The results from the optical appearance assay were in agreement with the microbial tests. The lightness (L*) values of the control samples were statistically significantly lower than those of the samples treated with chitosan solutions during storage. Additionally, the control samples exhibited higher b* values at the end of the storage period, indicating that untreated samples shifted toward a yellow color more rapidly than those treated with chitosan-based coatings.

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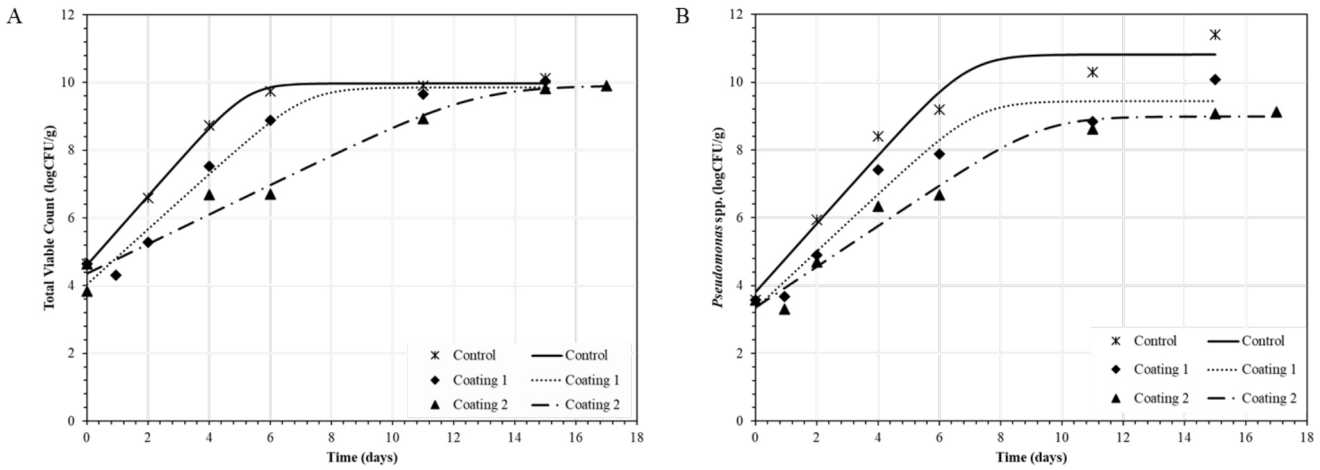


Figure 1 Microbial growth of (A) TVC; and (B) *Pseudomonas* spp. in gilthead seabream fillets stored isothermally at 2°C.

**Conclusion**

Chitosan based coatings are an effective packaging technique for extending shelf life and maintaining the quality of fresh fish fillets. Citrus extract can be successfully incorporated as an active compound in chitosan-based solutions, supporting sustainable food preservation practices.

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# EFFECTS OF NUTRIENT AND ELECTRICAL CONDUCTIVITY STRESS ON EVAPOTRANSPIRATION AND STOMATAL CONDUCTANCE IN LETTUCE (*Lactuca Sativa*) GROWN IN AQUAPONIC SYSTEMS

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## Introduction

Optimizing aquaponic production requires understanding how plants balance water loss and biomass accumulation under suboptimal nutrient and EC conditions. This study examines the physiological responses of lettuce to varying nutrient and EC levels in aquaponic systems to develop nutrient management strategies that enhance productivity and resource efficiency.

## Abstract

Understanding how plants regulate water loss and biomass under suboptimal nutrient and electrical conductivity (EC) conditions is key to optimizing aquaponic systems on the side of hydroponics plant production. This study examined physiological responses of lettuce (*Lactuca sativa* L. cv. Alyssa) grown in a nutrient film technique (NFT) system under six treatments varying nutrient concentrations (27%, 49%, and 76% of Sonneveld hydroponic solution) with or without EC control (~2.2 mS $cm^{-1}$). Treatments AP27 and AP49 (i.e., 27% or 49%, respectively) combined nutrient and fluctuating EC stress. Nutrients were supplemented at weeks 1 and 3, creating alternating phases of sufficiency and transient stress in weeks 2 and 4. Weekly evapotranspiration rate (E, mol $m^{-2}$ $s^{-1}$) and stomatal conductance to water vapour ($g_{sw}$, mol $m^{-2}$ $s^{-1}$) were measured with an IRGA gas analyser (Licor, LI-COR 6800). Linear mixed-effects models showed significant effects of treatment, week, and their interaction on E and $g_{sw}$. AP49 had higher E and $g_{sw}$ in week 1, reflecting higher stomatal activity under moderate nutrients without EC control, but these declined in weeks 2 and 4, aligning with nutrient stress periods. AP49fixed ("fixed" variant for controlled EC) showed consistently lower E and $g_{sw}$, indicating reduced physiological flexibility under fixed EC. Despite these shifts, AP49 achieved high biomass by week four, suggesting efficient resource use and partial decoupling of growth from water-use traits. The findings highlight that moderate, fluctuating nutrient regimes in aquaponics can boost productivity while conserving inputs and sustaining physiological resilience.

## Materials and methods

Lettuce (*Lactuca sativa* L., cv. Alyssa) was cultivated for 28 days in nutrient film technique (NFT) under six nutrient treatment regimes using hydroponic solution or decoupled aquaponic water (HP or DAPS). Each treatment had six independent replicates (gutters) with eight plants each. Plants 2 to 6 were sampled, with plants 1 and 8 as border plants. Evapotranspiration rate (E, mol $m^{-2}$ $s^{-1}$) and stomatal conductance for water vapour ($g_{sw}$, mol $m^{-2}$ $s^{-1}$) were measured weekly on the same plants in three replicates. To preserve plant identity for repeated measurements, destructive biomass sampling was performed weekly on the other three replicates. Nutrient concentrations were based on 76% Sonneveld solution (Sonneveld et al., 1999) for HP76fixed (control) and AP76fixed, and reduced to 49% and 27% in AP49fixed, AP27fixed, AP49, and AP27. Electrical conductivity (EC) was maintained between 2.2–2.3 mS $cm^{-1}$ in fixed treatments; EC fluctuated in AP49 and AP27. pH was maintained at 5.8 in all treatments.

## Statistical analysis

Fresh and dry weights were checked for normality and variance homogeneity, then compared across treatments weekly using one-way ANOVA with Tukey's post hoc tests based on three independent replicates per treatment for destructive sampling. Evapotranspiration (E) and stomatal conductance (gsw) were analysed using linear mixed-effects models with treatment, week, and their interaction as fixed effects, and plant ID as a random effect to account for repeated measures on three independent replicates used for non-destructive sampling. Degrees of freedom were approximated by the Satterthwaite method, and pairwise comparisons used Tukey adjustment. Analyses were performed in R version 4.4.1 (2024) using lme4, lmerTest, emmeans, and ggplot2. Significance was set at $\alpha < 0.05$.

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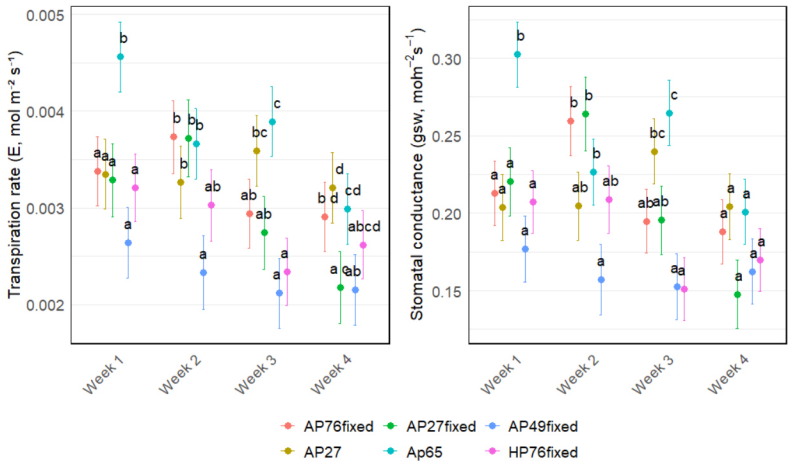


Figure 1: Weekly evapotranspiration rate (E, mol $H_2O$ $m^{-2}$ $s^{-1}$) and stomatal conductance ($g_{sw}$, mol $H_2O$ $m^{-2}$ $s^{-1}$) in lettuce across treatments. Values are estimated marginal means from linear mixed-effects models. Error bars show standard error. Letters indicate Tukey-adjusted significance groups ($\alpha = 0.05$), with comparisons made within each week.

**Results**

Significant effects of treatment and time were observed on evapotranspiration rate and stomatal conductance, with notable treatment × week interactions. The AP49 treatment exhibited higher E and $g_{sw}$ at week 1 compared to other treatments, indicating increased physiological activity under moderate nutrient levels without EC control. However, these values declined during weeks 2 and 4, coinciding with periods between nutrient applications and suggesting transient nutrient stress. In contrast, the AP49fixed treatment showed consistently lower E and $g_{sw}$, reflecting reduced physiological flexibility under fixed EC conditions (Figure 1). Despite these fluctuations, AP49 maintained high biomass accumulation by week 4, with significantly higher fresh weight (358.97 g ± SE 22.57) than AP76fixed ($p = 0.0188$), AP27fixed ($p = 0.0007$), and AP27 ($p = 0.0025$), indicating efficient resource use and partial decoupling of water-use traits from growth.

**Conclusions**

Moderate, fluctuating nutrient strategies combined with variable EC conditions can enhance stomatal regulation, water-use traits, and final biomass accumulation in aquaponic lettuce. The ability of plants to adjust transpiration and stomatal conductance under transient nutrient stress suggests potential for optimizing resource use without reducing growth. These findings support the use of dynamic nutrient management in aquaponic systems to balance productivity and sustainability.

**Acknowledgments**

This work is part of the Belmont Forum project within the SSCP2022 call AquaponicsOpti funded through the German Research Foundation (DFG) with grant number 516754420

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# USE of *Musa acuminata* AS AN ANTI-STRESSOR AND WATER FOULING REDUCTION IN CULTURED *Clarias gariepinus Burchell, 1822*

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Introduction
Water fouling reduction is needed in fish farming to reduce the number of times water is being changed. This study assessed the use of *Musa acuminata* as water fouling reduction and anti-stressor in cultured *Clarias gariepinus*.

Materials and Methods
Two hundred (200) fingerlings of *Clarias gariepinus* were purchased from LASU hatchery and stocked in a 500 litres tank for 3 weeks (21days) in order to induce stress on the fishes by overstocking, water was not changed and feeding was not regular and fed once per day.

After 3 weeks, the fishes were separated into two different tanks of 500 litre capacity each. Each tank (labeled 1-2) was stocked with 100 fishes each. For Tank 1- the control, no banana leaves were added while Banana leaves were inserted into tank 2 for a duration of two (2) weeks, The behavioural responses were recorded daily. Physical and chemical parameters of the water were recorded in the control and experimental tank containing banana leaves and later analyzed. The leaf extracts and the blanks were analyzed at the World Bank Step B Laboratory of the Federal Institute of Industrial Research, Oshodi, (FIIRO), Lagos State using the Atomic Absorption Spectrophotometer (SCHIMAZU-AA-7000).

**Table 1: Nutritional value of banana leaves**

| Parameters | Banana leaves | | |
|---|---|---|---|
| | $R_1$ | $R_2$ | Average |
| Carbohydrate | 85.15 | 85.10 | 85.13 |
| Protein | 3.67 | 2.68 | 3.18 |
| Fibre | 3.29 | 2.79 | 3.04 |
| Ash content | 1.18 | 1.26 | 1.22 |
| Moisture content | 7.01 | 7.00 | 7.01 |
| Fat content | 0.67 | 0.57 | 0.62 |

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Results

Average values for control and experimental tanks were Dissolved oxygen (5.70) mg/L, Carbon IV oxide (2.30) mg/L, Ammonia (0.50) mg/L, Temperature (27.72$^{o}$C) and pH (6.65). Dissolved oxygen (3.75) mg/L, Carbon-dioxide (3.66) mg/L, Ammonia (1.20) mg/L, Temperature (26.38$^{o}$C), pH (7.98) respectively. Mineral content of the tank with banana leaf had Ca(994.86)mg/L, Mg(515.46)mg/L, K(1558.22)mg/L, Na (21.40)mg/L, Fe(1418.05)mg/L, Zn(202.07)mg/L, Pb(0.007)mg/L, Cu(22.03)mg/L, Mn(188.09)mg/L and Ni(0.0075)mg/L. The average nutritional value of the banana leaf in the tank contained carbohydrate (85.13%), protein (3.18%), Fibre (3.04%), Ash content (1.22%), Moisture content (7.01%) and Fat content (0.62%). The values in the tank containing the banana leaves were found to be higher but within the WHO permissible limits. The high values indicate a high mineral, chemical and nutritional content than the control tank. The GC-MS revealed the presence of hexadecanoic acid, Tocopherol (vitamin E), phytol and phthalates compounds and other twenty (20) chemical compounds obtained in *musa acuminata*. These chemical composition influences fouling reduction, and reduction of stress- related diseases in *Clarias gariepinus*.

Conclusion

Phytochemicals present in banana leaf infuses into the water and induces water fouling reduction for 30 days in stressed *Clarias gariepinus* fish in culturing system.

# LAYING THE MUSSEL PUZZLE: LIFE CYCLE ENVIRONMENTAL IMPACTS OF MUSSEL FARMING PRACTICES ACROSS THE NORTHEAST ATLANTIC

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## Introduction

Farmed mussels have a special position among low-climate foods, much connected to their independence of manufactured feeds. This extensive and flexible production system of comparatively sustainable and nutritious food does not require specialised high-tech equipment and is therefore of special interest for a resilient food supply. The Mussel Puzzle is a three-year (2024-2026) research project, quantifying life cycle environmental impacts of farmed blue mussels in locations across the North Atlantic. One goal of the Mussel Puzzle is collecting and analysing systematic life cycle data for different forms of mussel farming. Additional goals are identifying improvement options in each system and evaluating what a large-scale expansion of production would mean in each location. Here we present first results for the life cycle environmental impacts of mussel farming using different techniques in three countries.

## Methods

We used Life Cycle Assessment (LCA) methodology to collect and analyse data on resource use and three categories of environmental impacts of mussel farming: greenhouse gas emissions, marine eutrophication potential and land use, quantified per tonne of liveweight mussels harvested. Note that land use only includes use of land area and not sea area. These three life cycle impact categories are expected to provide a useful, and potentially contrasting, picture of the environmental impact of farmed mussels, although the more local ecological potential impacts are not covered. The dependency on agricultural land use is, however, often used as an indicator of terrestrial biodiversity impacts. Invitations to participate in the project were sent out widely to mussel producers in all countries bordering the North Atlantic. To those that showed interest and willingness to share data, a data collection spreadsheet was sent which was followed up by online or face-to-face meetings, in which the production technique and the composition of a typical mussel rig in terms of material use and lifetime were discussed and quantified, as well as boat use and the fate of the biomass harvested. Data was collected for the period 2021-2024, indicating average, maximum, and minimum production conditions during that period. In this study, we want to analyse the relative contribution of the production of the mussel rig (i.e. the infrastructure) compared growout to total environmental impacts of on three farm sites across the Northeast Atlantic, using different farming technologies and strategies.

## Results

The length of the farming cycle of blue mussels differs between 10 and 24 months in the three locations analysed here (Table 1). Some producers let mussel spat settle naturally and let them grow until harvest size, others harvest and reseed the mussels on ropes after 3-4 months, to achieve higher growth, and more even size and quality of the mussels harvested. Fuel use was between 17-35 l/tonne mussels harvested, depending on the type and size of vessels used, whether mussels are only harvested once or twice to be reseeded, and the area over which each producer operates. On average, infrastructure stands for 11-54% of greenhouse gas emissions, 1-58% of marine eutrophication and 1-62% of land use (Fig. 1). Land use is dominated by the farming of cotton used in the cotton sock when reseeding as well as fuel production. Eutrophication is likewise dominated by manufacture of the cotton sock. Greenhouse gas emissions are instead dominated by fuel production and combustion.

## Discussion

Early results show that there are big differences in life cycle environmental impacts between and within locations and farming techniques, which opens up for improvements of already low-impact production systems. Fuel use of boats used in growout is the single most important contributor to greenhouse gas emissions in all systems, so efforts to reduce the fuel use, and shifting to alternative fuels, would help reducing farmgate emissions. One way to reduce the need for using boats, is if the rigs could be monitored digitally, and this kind of technology is currently being developed for low-trophic aquaculture. While there are indications that reseeding comes at the cost of higher fuel use (Frösell 2019), we cannot draw this conclusion here, since there are too many other factors that influence the fuel use in mussel farming. In coming years, data will be complemented, and the possibilities and tradeoffs connected to large-scale expansion of mussel farming will be mapped. If you are interested in collaborating, as a producer, researcher, or have questions about the project – feel free to get in touch!

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**Table 1** Key aspects of the three production sites, described by production technique and ocean area

| Environmental indicator | Natural settling Skagerrak | Reseeding Kattegat | Reseeding North Atlantic |
|---|---|---|---|
| Length of production cycle (months) | 15-24 | 10-16 | 18-24 |
| Fuel use (l/tonne mussels harvested) | 35 | 17 | 22 |
| Average annual production (tonnes) | 1700 | 1400 | 800 |

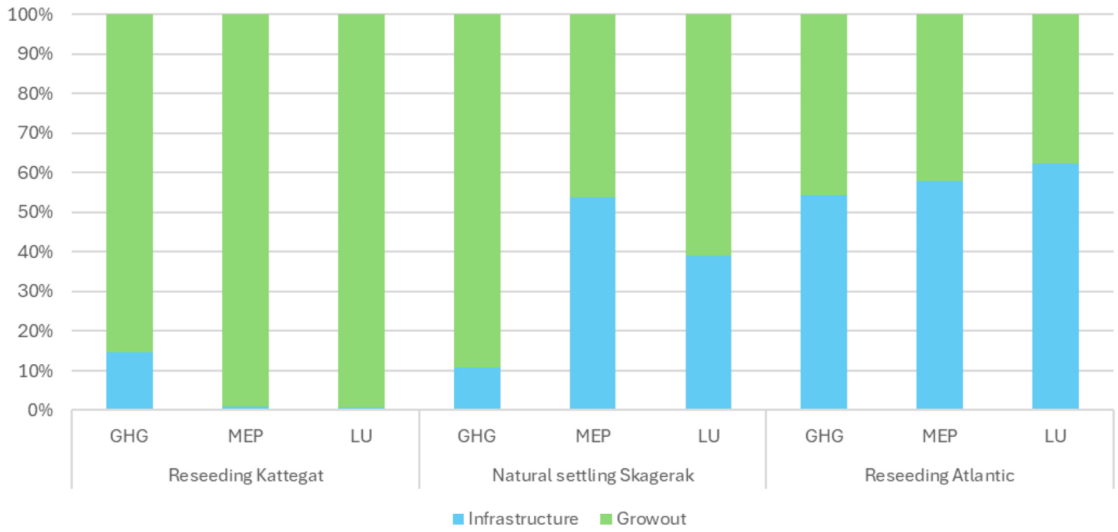


**Fig. 1** Proportion contributed by infrastructure vs growout, where growout means fuel production and consumption and supply materials with a life-time shorter than one year and infrastructure the production of materials with a lifetime of one year or longer.

**Reference**

Frösell, M. (2019) Mussel farming using various techniques evaluated using Life Cycle Assessment (LCA) BSc thesis SLU, Uppsala (Available online: https://stud.epsilon.slu.se/14639/, Last accessed April 8, 2025)

# FISH BRAIN-GUT AXIS – BRAIN RESPONSIVENESS TO INTESTINAL INFLAMMATION

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## Introduction

Similar to what is witnessed in mammals, teleost fish present a network of immune, neural and endocrine signals enabling a delicate and efficient bidirectional communication between the central nervous system and the gastrointestinal tract. Among other processes, intestinal inflammation triggers a cascade of events that necessarily lead to this brain-gut axis activation, which will then unfold multiple regulatory mechanisms. In fish however, such intricate processes are poorly documented. Aiming at using fish brain-gut axis as an asset in nutritional health-enhancing strategies, it is imperative to fully grasp its most intrinsic dynamics. This preliminary approach intends to evaluate the outreach of a peripheral inflammatory process (enteritis) in activating central neuroendocrine mechanisms, focusing on gene expression changing patterns at both ends of the brain-gut axis

## Material and Methods

Juvenile rainbow trout (RT, 45 ± g), *Oncorhynchus mykiss*, were distributed in two, 300 l tanks, one group being fed a commercial diet and the other group the same commercial diet supplemented with dextran sodium sulfate (DSS, 1% dry matter) to induce acute enteritis. Fish were fed twice a day *ad libitum*, and were sampled at the end of 5- and 15-days feeding periods. Samples of hypothalamus, pituitary gland and anterior intestine were collected and processed for RNA extraction. A panel of immune and neuroendocrine molecular markers was carefully assembled and gene expression was measured by real time PCR. Moreover, transcriptomic (RNAseq) analysis was performed in the anterior gut samples by Illumina sequencing.

## Results

The expression level of immune-related genes such as cluster of differentiation 3 and 8 (*cd3* and *cd8*), aryl hydrocarbon receptor (*ahr*) and interleukin 1β (*ilβ)* was upregulated in RT gut fed DSS-supplemented diets for 15 days. Expression of the serotonin transporter *serta* was similarly increased in this group and tissue, and so was gene expression of the transmembrane protein *claudin*. Hypothalamic corticotropin-releasing hormone (*crh*) expression increased in fish fed DSS-supplemented diet for 15 days. No significant changes were observed among genes studied in the pituitary gland. In what the transcriptomic approach is concerned, while 5 days of DSS-feeding only slightly changed gene expression (a total of 22 differential expressed genes, DEGs), a 15-days feeding period more significantly affected gut transcriptomics (120 DEGs). Regarding the longer feeding period, the biological function digestion was enriched by several downregulated genes of enzymes such as trypsin and carboxypeptidase. In contrast, immune-related terms such as leukocyte proliferation and T-cell activation were found enriched by upregulated genes such as TSC22 domain family protein 3 isoform X1 (*tsc22*), Kruppel like factor 9 (*klf9*) and transforming growth factor beta activator (*lrrc3*).

## Discussion

The enhancement of immune cell markers (*cd3* and *cd8*) and inflammatory mediators (*il1β* and *ahr*) observed by real-time qPCR in the target tissue of the inflammatory model – the intestine – points to the successful implementation of an inflammatory dietary compound typically used in mammalian studies. As somewhat expected, serotonin dynamics seemed to be altered as well as denoted by the upregulated expression of its transporter. Such peripheral serotonergic changes stand as potential triggering mechanisms of central neuroendocrine activity. Indeed, at the hypothalamus level, expression levels of the first secreted hormone (CRH) were enhanced, representing a strong indication of the brain responsiveness to intestinal inflammatory conditions. In accordance with real-time qPCR analysis, intestine RNA sequencing of fish fed DSS-supplemented diet for 15 days depicts a state of immune activation and compromised digestion. However, no indication of changes to serotonin-related pathways was found among the observed DEGs.

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In conclusion, results point to DSS dietary administration as a promising enteritis model in RT. Despite no changes in serotonin dynamics were observed during the RNA-sequencing analysis in gut samples, the increased expression of related genes in both gut and brain tissues is an indication of brain responsiveness to gut inflammation.

**Acknowledgments**

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# EFFECT OF ELECTRIC SHOCK ON GROWTH AND SEX REVERSAL IN RAINBOW TROUT (*Oncorhynchus mykiss*, WALBAUM, 1792)

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## Introduction

Sex control and thus the understanding of sex determination and sex differentiation in fishes is crucial in aquaculture due, for example, to the need for controlling reproduction, producing monosex populations. The production of such populations is of particular importance owing to differences in growth rates or economical values of sexes in some species (Yamazaki, 1983). Various methods, including hormonal treatments, chromosomal manipulation, hybridization, and selective breeding, have been employed to achieve sex control in fishes (Wang et al., 2018). Besides these techniques, some studies reported that the use of electric shock can also influence sex ratios. For instance, Okomoda et al. (2020a) showed that applying electric shock to red hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) resulted in a 70% male population. Electric shock has been widely used for triploid production in various species, including mussels (Cadorel, 1992), African catfish (*Clarias gariepinus*) (Okomoda et al., 2020b), tilapia (Hassan et al., 2018; Okomoda et al., 2020a), and coho salmon (*Oncorhynchus kisutch*) (Teskeredžić et al., 1993). The main objective of our study was to investigate the effects of an electric shock on the development of embryos of rainbow trout gonads varying the timing of the shocks after fertilization while avoiding the production of triploid populations. To do so we measured sex ratio and quantified, growth performance, and fatty acid composition that ultimately will affect the nutritional values for consumers.

## Materials and methods

Three male and five female rainbow trout (*Oncorhynchus mykiss*) were used for gametes collection. Fertilized eggs of rainbow trout were exposed to a direct electrical current of 12V for 10 minutes at different times post-fertilization (20, 30, and 40 minutes), using well water with different salinity levels (5ppt and 0ppt). The electric field was generated using a rechargeable battery connected to rectangular aluminium electric probes, placed 30 cm apart to match the container's cross-sectional area within a plastic container of 35x25x15cm. Nitrate, chloride, and carbonate/bicarbonate were also tested using standard methods. Analysis for potassium, magnesium, sodium, cobalt, barium, beryllium, bismuth, selenium, chromium, iron, nickel, copper, and calcium was performed following Method 200.7. Erythrocyte measurements were analysed to assess ploidy levels. Growth performance, live weight gain, survival rate, total feed intake, feed conversion ratio, and specific growth rate were calculated. Histological procedures were performed to identify the cell types involved in spermatogenesis or oogenesis; samples were stained with Hematoxylin-Eosin. The sex ratio was determined by histological examination of the gonads, with groups categorized based on electrical exposure post-fertilization. Fatty acids analysis was conducted in muscle and liver tissue using Gas Chromatography.

A two-sample t-test was used to compare fertilization rates between treatments. Fertilization and hatching rates, water parameters, growth performance, and fatty acid profiles were analysed using one-way ANOVA, followed by Tukey's post hoc test ($P < 0.05$). Ploidy determination was conducted using one-way ANOVA with Fisher's post hoc test ($P \leq 0.05$). All statistical analyses were performed using Minitab 18. Principal component analysis of erythrocytes was carried out in RStudio.

## Results

The results of the application of an electric shock at different post-fertilization times (20, 30, and 40 minutes) and salinity levels (0 and 5 ppt) did not significantly affect the fertility or hatching rates. Ploidy determination showed significant differences among treatments ($P \leq 0.05$); however, the values are consistent within the diploid range, reinforcing the conclusion that the observed characteristics are typical of diploid organisms. Groups shocked at 0 ppt salinity after 20-, 30- and 40-minutes fertilization showed greater values of cell-nuclear area ratio and cell-nuclear volume ratio to Principal Component 2, where the nuclear structure may be more affected by electric shock. Triploidy was not observed, as triploid individuals exhibited significantly larger cell and nuclear dimensions. Initial weights did not significantly differ among treatments ($P > 0.05$). However, significant differences were observed in final weight and total weight gain ($P < 0.05$). The highest final weight was

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recorded in the at 0 ppt salinity after 30 minutes fertilization (39.07 ± 0.08g), which was significantly higher than most other treatments. The sex ratio results showed no significant differences among the experimental groups or the control group ($P > 0.05$). Histological examination of gonads from the experimental groups and the control group showed no differences between the experimental and control groups regarding gonadal structure, both in males and females. No significant differences were observed in the overall proportions of saturated, monounsaturated, polyunsaturated, or total trans fatty acids in both muscle and liver, indicating that the electrical shock treatments did not alter the global balance of fatty acid groups. However, linoleic acid (C18:2) was significantly higher in the 20 minutes post-fertilization group compared to the control. Similarly, linolenic acid (C18:3) was significantly elevated in the 20- and 30-minutes post-fertilization groups relative to the control. Eicosapentaenoic acid (EPA, C20:5) was also significantly higher in the 30 minutes post-fertilization group compared to the control.

**Discussion and conclusions**

This study demonstrates that exposure to a 12V (voltage gradients 0.4 V/cm) for 10 minutes at different times (20, 30, and 40 minutes) after fertilization have no negative effects on fertility and hatching rates in rainbow trout embryos. Despite the relatively low voltage gradient, 12V electrical shock still induced significant developmental impacts, reinforcing the sensitivity of embryos during early developmental stages. No triploidy induction was confirmed, as erythrocyte characteristics remained consistent with diploid individuals. However, 12V electrical shock influenced growth performance and showed differences in specifics fatty acid profiles, possible due to metabolic stress rather than ploidy-related effects. Future research should investigate the combined effects of a 12V electrical shock with other environmental factors, such as temperature, oxygen levels, or salinity, to better understand their interactions and potential applications in aquaculture.

**Acknowledgments**

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# STATISTICAL PREDICTION OF FERTILIZATION, HATCHING AND EYED-STAGE DEVELOPMENT IN RAINBOW TROUT (*Oncorhynchus mykiss*) FED WITH PROBIOTIC-ADDITIVE DIET

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## Introduction

Reproductive success is a key determinant in aquaculture productivity, particularly in species of high commercial value such as rainbow trout (*Oncorhynchus mykiss*). In recent years, dietary probiotics have gained attention as promising functional feed additives to enhance reproductive parameters through improved immunity, nutrient absorption, and overall broodstock health (Abkari-Nargesi et al., 2020). Among the microbial candidates, *Lactococcus lactis* (Abkari-Nargesi et al., 2020) and *Hafnia alvei* (Didinen et al., 2016) have shown beneficial effects in various animal models. However, their specific influence on fish gamete quality and early embryonic development remains underexplored. In recent years, statistical modelling has been used in aquaculture to predict outcomes of dietary additives on growth and feed efficiency (Serradell et al., 2023; Qian et al., 2024). Statistical prediction based on fertilization outcomes offer a valuable statistical framework to estimate reproductive outcomes, such as hatching and eyed-stage success. This study aims to statistically evaluate the predictive power of fertilization rate on embryonic development stages under different probiotic treatments, using linear regression models.

## Materials and methods

This study was conducted using data from an experimental study of the project supported by TUBİTAK (Project No:222N019) to evaluate the effects of probiotic supplementation on gamete quality in rainbow trout (*O. mykiss*). The experimental diets were supplemented with two different probiotic bacterial species, each at two different concentrations. The probiotic bacteria used in the study were *Hafnia alvei* (P10) and *Lactococcus lactis* (P9), at two concentrations $10^7$ (C1) and $10^9$ (C2) CFU $g^{-1}$. First, the effect of the two different bacterial species was analysed regardless of their concentration. Second, the effect of the bacterial concentration was evaluated regardless of the bacterial species. Fertilization, eyed-staged and hatching rate were measured for a simple linear regression model in order to analyse the relationship between fertilization rate (independent variable) and either eyed-stage or hatching rate (dependent variable). The analyses were performed using RStudio 4.4.1. The outputs included predicted values, 95% confidence intervals, correlation coefficient (R) and coefficient of determination ($R^2$), where the formula (*Estimated outcome* = $\beta 0 + \beta 1$ *x Fertilization Rate*). These metrics were used to evaluate the predictive strength of fertilization rate on eyed-stage and hatching success, and to compare model performance across different probiotic treatments.

## Results

The fertilization and eyed-stage rate parameters for rainbow trout used in the experiment showed the following correlation coefficients: 0.99 for the low-concentration C1 group and 0.95 for the high-concentration C2 group. Coefficients of determination ($R^2$) were found to be 99.93% for the C1 group and 90.76% for the C2 group. The fertilization and hatching rate parameters showed the following correlation coefficients: 0.77 for the low-concentration C1 group and 0.79 for the high-concentration C2 group. Coefficients of determination ($R^2$) for the fertilization rate and eyed-stage rate were found to be 55.95% for the C1 group and 62.79% for the C2 group. By using different probiotic bacterial strains, the correlation coefficients between fertilization and eyed-stage rates were 0.99 for the P10 bacteria group and 0.93 for the P9 group. The determination coefficients were 99.58%

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for P10 and 86.72% for P9. The correlation coefficients between fertilization and hatching rates were 0.75 for the P10 bacteria group and 0.79 for the P9 group. The determination coefficients were 55.78% for P10 and 66.06% for P9.

**Discussion and conclusions**

This study has focused on statistical predictions of the effects of probiotics additives on gamete quality of rainbow trout. Our results suggest that while the lower dose (C1) supports more consistent embryonic development (eyed-stage), the higher dose (C2) may be more effective in enhancing hatching success. Mathematical modelling has been recognized as an effective method for evaluating the relationship between physiological and environmental parameters in fish, considering different variables (Qian et al., 2024). Serradell et al. (2023) have reported that using a multiple linear regression model in *Dicentrarchus labrax*, probiotics added to feed explained 67%, 80%, and 63% of the variation ($R^2$) in specific growth rate (SGR), feed conversion ratio (FCR), and feed intake (FI), respectively. However, the model showed that the probiotics obtained values did not have a significant influence on the predicted outcomes. In this study, statistical analyses indicate that P9 has a higher predictive association with hatching success, while P10 showed a more consistent predictive relationship with the development of embryos to the eyed stage. These patterns were also supported by high correlation and determination coefficients, particularly for the fertilization and eyed-stage rates in the C1 and P10 groups. Furthermore, statistical prediction confirmed the stronger reliability of these relationships in lower probiotic concentrations and with the P10 strain, supporting their potential for improving reproductive success in rainbow trout.

**Acknowledgments**

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# THE ROAD TO BECOMING THE WORLD'S LEADING *Acartia tonsa* PRODUCER

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## Introduction

Plankton production, particularly copepods (*Acartia tonsa*), represents one of aquaculture's most complex biological challenges. Their fragility, sensitivity to environmental change, and complex life cycle have made industrial-scale production difficult to achieve.

## From Start-up to Industrial Scale

After several years of persistent effort, CFEED has achieved robust, industrial production of copepods. Transforming from a promising start-up into the first large-scale copepod producer required more than simply scaling laboratory processes. Achieving consistent product quality and yield at a commercial scale demanded careful optimization of production conditions and parameters. This presentation will chronicle our journey: from the initial scientific and operational challenges in mass copepod cultivation, through strategic pivots that redefined our processes, to the key technological innovations that enabled us to scale efficiently, sustainably, and with strong biosecurity.

## Overcoming Market and Regulatory Challenges

We will share how we overcame significant market entry barriers, such as ensuring consistent biomass quality and meeting strict regulatory standards, while building a resilient company culture based on science and environmental stewardship. Our approach leverages key innovations: advanced bioreactor technology, controlled production systems, a newly designed reproduction program, and data-driven optimization to streamline operations and minimize environmental impact.

## Environmental Impact and Feed Efficiency

A central outcome of these innovations is the long-term improvement of feed conversion ratio (FCR) in aquaculture species, reducing the overall need for feed. This is especially significant because approximately 70% of aquaculture's global $CO_2$ emissions are linked to feed production. By improving FCR and lowering feed demand, our solutions directly contribute to reducing the carbon footprint of the industry and advancing more sustainable aquaculture practices.

## Conclusion and Insights

Attendees will gain actionable insights into the critical success factors behind long-term scaling in the blue biotech sector, including continuous innovation. Our story offers valuable lessons for organizations seeking to break through growth plateaus and achieve lasting impact in sustainable aquaculture. By sharing our experience, we aim to contribute to the ongoing dialogue on sustainable plankton production and provide examples for others facing similar scaling challenges.

# BIVALNET: NETWORK FOR THE STUDY AND PROMOTION OF THE ECOSYSTEMIC SERVICES OF BIVALVES

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BIVALNET is a research network established in 2023, dedicated to study and promote the ecosystem services provided by bivalves. Coordinated by the IIM-CSIC, the network brings together researchers from the University of Cádiz (UCA), University of Vigo (UVIGO), University of the Basque Country (UPV/EHU), the Oceanographic Centres of Murcia, Cádiz, and Vigo (IEO-CSIC), the Torre de la Sal Aquaculture Institute (IATS-CSIC), and the Institut de Ciències del Mar (ICM-CSIC).




Figure 1. Map showing the institutions that are currently part of Bivalnet network.

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The objectives of BIVALNET include, among others: (i) fostering long-term collaboration among the network's research groups; (ii) integrating scientific knowledge across disciplines to enhance the understanding and promotion of bivalve ecosystem services; (iii) establishing protocols for joint use of research equipment and infrastructure; (iv) promoting the mobility of trainees and early-career researchers; (v) facilitating knowledge transfer between research groups and supporting the development of new collaborative projects; (vi) raising public awareness about the goods and services provided by bivalves; (vii) encouraging the advancement and dissemination of bivalve-related research; (viii) supporting public policy development that benefits bivalve aquaculture and the restoration of bivalve populations; and (ix) strengthening relations with international organizations focused on the recovery of wild bivalve populations and their associated ecosystem services.

Several groups within the network are actively engaged in research on the use of the flat oyster (*Ostrea edulis*) for bioremediation and restoration purposes. In addition, BIVALNET seeks to broaden its scope by integrating knowledge on other bivalve species and involving research groups not yet part of the network but who share its core objectives. This expansion aims to strengthen multidisciplinary collaboration and advance the collective understanding of the ecosystem services provided by bivalves.

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Key words: ecosystem services, network, bivalves

# MONITORING ENVIRONMENTAL INTERACTIONS OF MARINE AQUACULTURE IN ALGERIA: INSIGHTS FROM THE DZAQUAFARM PROJECT

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Marine aquaculture in Algeria is experiencing rapid growth, driven by increasing demand for seafood and the need to diversify economic activities in coastal regions. However, this expansion poses significant environmental challenges, including water quality degradation, habitat alteration, and interactions with marine ecosystems. The DZAquaFarm project addresses these challenges by implementing an integrated environmental monitoring system for marine aquaculture sites across Algeria.

The project combines in-situ measurements and remote sensing data to monitor key environmental parameters, such as oceanographic conditions, phytoplankton dynamics, and ecotoxicological indicators. These data are integrated into a Geographic Information System (GIS) and visualized through an interactive webmapping platform. This system enables real-time monitoring of aquaculture sites and provides stakeholders with actionable insights into the environmental status of marine farms.

Preliminary results highlight the variability of environmental conditions across aquaculture sites, with some areas showing signs of eutrophication and increased susceptibility to harmful algal blooms. The project also identifies sensitive coastal zones where aquaculture activities may exacerbate anthropogenic pressures. A classification system has been developed to label aquaculture farms based on their environmental performance, promoting best practices and sustainable management.

By providing a comprehensive understanding of the interactions between aquaculture and the marine environment, the DZAquaFarm project supports evidence-based decision-making for sustainable aquaculture development. The platform also serves as an early warning system for environmental risks, such as toxic phytoplankton blooms, enhancing the resilience of the aquaculture sector in Algeria. This approach demonstrates the importance of integrated monitoring systems in balancing economic growth with environmental conservation in Mediterranean coastal regions.

# MEDITERRANEAN PRAWN (*Penaeus kerathurus*) AS POTENTIAL EXPLOTAIBLE SPECIES IN MEDITERRANEAN AQUACULTURE

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**Introduction**

According to the European Union (EU) strategic guidelines (COM/2021/236 final), the long-term sustainability of the aquaculture sector passes through both the diversification of species and implementation more environmentally friendly systems. Thus, the share of farmed crustacean has risen over time and nowadays they are in second position of the global trade in aquatic animal products in 2022, making up 23 percent of the total value (FAO, 2024). *Penaeus (Melicertus) kerathurus* (Forskål, 1775) is a benthic species native to the Mediterranean Sea and the eastern Atlantic Ocean, with a distribution range extending from the coasts of Portugal to Angola (Zitari-Chatti et al., 2008). During the 1970s, *P. kerathurus* was considered a promising candidate for intensive aquaculture in southern Europe, however, several issues reduced its commercial exploitation in favor of other species. Despite its economic and ecological significance, knowledge regarding the species' biology remains limited, and data on population structure are scarce. This lack of information hinders the development of effective cultivation methods for commercial purposes. The present study seeks to expand our understanding of the biology and ecology of *P. kerathurus*, with the aim of establishing an optimal and economically viable aquaculture methodology.

**Materials and Methods.**

In this work, a comprehensive literature search about *P. kerathurus* was conducted, focusing on enabling its cultivation under controlled conditions and ultimately achieving full life cycle closure in captivity. In addition, data from both extractive fishing activity and fattening practices in recent decades were obtained from the official website of the Ministry of Agriculture, Fisheries and Food of the Government of Spain. Literature review carried out using the electronic databases Scopus and PubMed to identify relevant scientific publications on the biology and ecology of the species. For this, *'Penaeus (Melicertus) kerathurus'* AND *'aquaculture'* were used as keywords, restricting publication time between 1980 and 2024. Initial screening focused on studies addressing the species biology and ecological characteristics. Subsequently, a more refined search was conducted to identify literature related specifically to breeding techniques and captive rearing practices attempted to date. Articles were selected based on their relevance to the scope of the review, prioritising those that presented empirical findings, practical methodologies, or reviews of aquaculture strategies applicable to the target species.

**Results and Discussion**.

At present, the culture of *P. kerathurus* in countries such as Spain is confined to the fattening phase within coastal lagoon systems (Figure 1).

Studies confirm that specific environmental conditions are required, such as a salinity range between 28–35 for reproduction and larval development, although standardised protocols for captive breeding remain underdeveloped. Experimental trials have achieved partial success in broodstock conditioning and early-stage larval rearing, but survival rates remain inconsistent. Major limitations include the scarcity of long-term studies and fragmented data across regions. Despite these gaps, current evidence supports the feasibility of closing the species' life cycle in captivity with further targeted research. Contemporary technological advancements in system design and intra-family nutrition (FAO, 2022) present renewed opportunities to critically reassess its agricultural potential.

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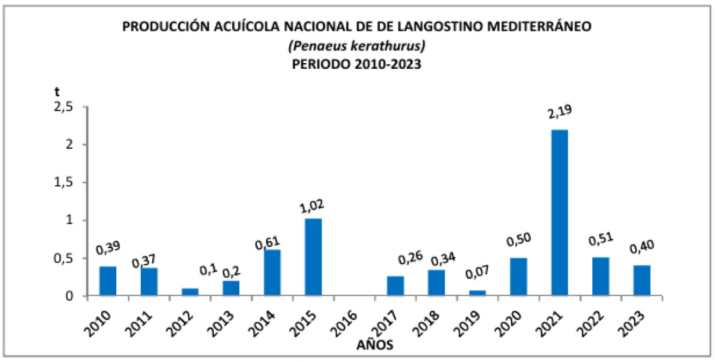


Fig 1. Aquaculture production of *P. kerathurus* in Spain (tonnes per year), based on public data from the official website of the Spanish Ministry of Agriculture, Fisheries and Food.

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# INTERACTIVE EFFECTS OF SALINITY AND DIETARY IRON ON ω3 LC-PUFA BIOSYNTHESIS IN *Platynereis dumerilii*

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**Introduction**

Polychaetes are promising sources of ω3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) due to their ability to synthesise these essential nutrients through the action of fatty acyl elongases (Elovl), front-end desaturases (Fed), and methyl-end desaturases (ω-des) (Monroig et al., 2022). It has been suggested that salinity can influence lipid metabolism in aquatic invertebrates, as it triggers osmotic stress that alters membrane lipid composition (Palacios and Racotta, 2007). Iron also plays a crucial role in lipid metabolism and, among other functions, iron is a cofactor of desaturase enzymes like Fed and ω-des involved in LC-PUFA biosynthesis (Shanklin et al., 2009). We hypothesize that salinity conditions leading to increased expression of Fed and/or ω-des will only result in increase desaturase activity and ultimately LC-PUFA biosynthesis, if iron supplementation ensures full activation of the desaturase enzyme pool. To test this hypothesis, this study investigated (i) the effects of salinity on fatty acid profiles and expression of genes involved in the ω3 LC-PUFA synthesis in the marine polychaete *Platynereis dumerilii*, and (ii) the combined effects of salinity and dietary iron on the *P. dumerilii* LC-PUFA biosynthesis.

**Materials and Methods**

Two *in vivo* trials were conducted using *P. dumerilii* juveniles maintained under standard conditions (18 °C, 16L:8D photoperiod; low-PUFA diet). Trial 1 assessed the effects of three salinity levels (30‰, 35‰, and 40‰), using three plastic boxes per salinity level, each containing 16 worms. In Trial 2, *P. dumerilii* were reared under four conditions combining two salinities (35‰ and 40‰) and two diets (with or without iron supplementation), using four plastic boxes per condition, each with 25 worms. At the end of each trial, samples were collected to evaluate the effects on the ω3 LC-PUFA biosynthesis through fatty acid profiling, and gene expression analysis of key desaturases and elongases of the *P. dumerilii* LC-PUFA biosynthesis. Principal Component Analysis (PCA) was conducted to explore patterns in fatty acid profiles and assess potential differences among treatments.

**Results**

For Trial 1, *P. dumerilii* exhibited elevated levels of ω3 LC-PUFA, specifically eicosatetraenoic acid (20:4n-3), eicosapentaenoic acid (20:5n-3), and docosapentaenoic acid (22:5n-3) at 40‰ salinity, compared to individuals maintained at 35‰ and 30‰, despite receiving the same diet. Gene expression analysis revealed a significant upregulation of the *P. dumerilii* Δ6/Δ8 Fed desaturase under high salinity (40‰), accompanied by a decrease in its metabolic substrates (20:2n-6 and 20:3n-3); these results indicate an enhanced desaturase activity at high salinity. In Trial 2, PCA of the fatty acid profiles showed a clear separation among the four experimental groups. The first component distinguished salinities, and the second separated diets with and without iron supplementation, especially at 40‰ salinity (Fig. 1). No effects from iron supplementation were observed at 35‰ salinity. Notably, at 40‰, iron supplementation correlated with increased levels of 20:3n-6 and 20:4n-3 (Fig. 1), both desaturase products from the *P. dumerilii* Δ6/Δ8 Fed and therefore supporting enhanced desaturase activity. These results support the conclusion that the ω3 LC-PUFA biosynthesis in *P. dumerilii* is influenced by both salinity and dietary iron. The proposed mechanism involves an upregulation of fatty acyl desaturases at high salinity, while iron supplementation guarantees the enzymatic activity that leads to enhanced LC-PUFA biosynthesis and improved nutritional value.

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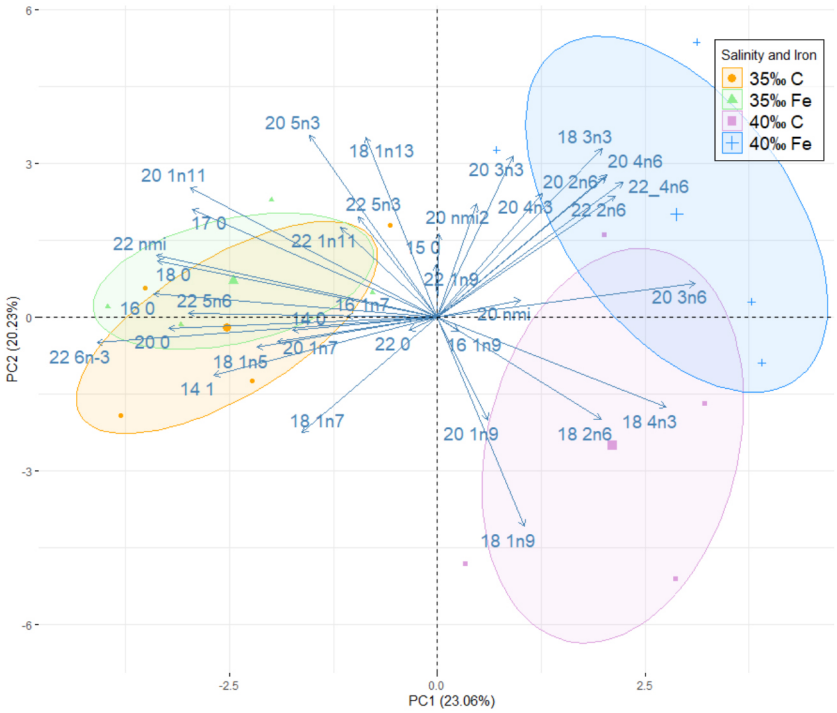


**Figure 1**. PCA of fatty acid composition in *P. dumerilii* under four treatments: 35‰ without iron (orange circles), 35‰ with iron (green triangles), 40‰ without iron (violet squares), and 40‰ with iron (blue crosses). Vectors represent fatty acids contributing to group separation; ellipses show 95% confidence intervals.

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## Acknowledgements

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# BY-PRODUCTS VALORIZATION OF AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822)

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## Introduction

In the context of advancing a blue and circular economy, utilizing the full potential of African catfish (*Clarias gariepinus* Burchell, 1822) by-products—specifically the liver, gonads, and skin—can significantly reduce waste and enhance resource efficiency. While considerable research has focused on the nutrition (Langi et al. 2024) and nutritional content of the fillets, there remains a notable gap in understanding the potential applications of the catfish's liver, gonads, and skin. Research shows that the liver of African catfish is rich in polyunsaturated fatty acids (PUFA) and has low concentrations of triglycerides and saturated fatty acids (SFA), particularly when the fish is fed high-protein diets (Matter et al. 2004, Taiwo et al. 2014).

The study aimed to unravel the composition of fatty acids of by-products of the African catfish within a Recirculating Aquaculture Systems (RAS) and the most suitable moment for harvesting to maximize the use of nutrients and their further potential reuse.

## Material and methods

We monitored the growth in total body weight, the hepatosomatic index (HSI), the gonadosomatic index (GSI), the skin weight and the filet weight (without the skin) of African catfish. Furthermore, the content of fatty acids in the liver, gonads and skin was analysed.

## Results

**Table 1.** Total body weight, HSI, GSI, skin and meat percentages over the total body weight of African catfish at 5 different samplings (each 3 weeks) in RAS

| | Sampling | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| **Total body weight (g)** | Female | 646.7 ± 52.8 | 1296.7 ± 221.8 | 1583.3 ± 187.7 | 1840.0 ± 331.8 | 2080.0 ± 391.5 |
| | Male | 641.0 ± 81.8 | 1323.3 ± 20.8 | 1597.3 ± 310.3 | 1753.3 ± 305.6 | 2223.3 ± 791.8 |
| **HSI (%)** | Female | 1.1 ± 0.3 | 1.1 ± 0.3 | 1.2 ± 0.1 | 1.1 ± 0.1 | 1.0 ± 0.2 |
| | Male | 1.9 ± 0.5 | 1.6 ± 0.1 | 1.9 ± 0.3 | 1.8 ± 0.2 | 2.2 ± 0.3 |
| **GSI (%)** | Female | 1.7 ± 1.2 | 3.2 ± 2.7 | 7.6 ± 2.6 | 13.5 ± 3.3 | 16.2 ± 3.2 |
| | Male | 0.4 ± 0.2 | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.9 ± 0.3 | 0.9 ± 0.3 |
| **Skin percentage (%)** | Female | 8.4 ± 1.1 | 6.9 ± 1.0 | 6.6 ± 0.3 | 9.1 ± 1.7 | 4.8 ± 0.7 |
| | Male | 6.2 ± 0.1 | 6.9 ± 1.1 | 7.3 ± 0.1 | 10.2 ± 0.4 | 6.5 ± 0.4 |
| **Meat percentage (%)** | Female | 41.9 ± 2.9 | 33.6 ± 6.4 | 38.8 ± 2.3 | 32.5 ± 3.8 | 34.2 ± 4.7 |
| | Male | 38.7 ± 0.5 | 24.0 ± 2.4 | 38.7 ± 3.5 | 40.9 ± 0.6 | 40.7 ± 3.7 |

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# LIGHT SPECTRUM, TEMPERATURE AND FEEDING REGIME EFFECT ON RAINBOW TROUT (*Oncorhynchus mykiss*): WELFARE AND PHYSIOLOGICAL RESPONSES

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## Introduction

Rainbow trout (*O. mykiss*) production in Europe accounts for approximately 20% of global production, making it the most farmed freshwater species. To optimize both economic efficiency and animal welfare, three key parameters were studied: temperature, light spectrum, and feeding regime. Temperature is a crucial factor affecting fish metabolism, growth, respiration, stress, and reproduction [1]. Light spectrum, as previous studies indicate that blue and green wavelengths can optimize growth performance in some teleosts [2,3]. Feeding regime is another vital parameter. A fixed feeding schedule that allows fish to anticipate food arrival, can impact growth and metabolism [4]. Proper feeding practices ensure that the fish receive adequate nutrition and maintain optimal health.

## Materials and methods

Juvenile trout (3,7±0,3 g weight and 6,7±0,2 cm length) were homogeneously distributed into eight independent experimental groups by duplicates, with different rearing conditions by combining different temperatures (15 or 20ºC), light spectrum [white 380-750nm (W); green 495-570nm (G); or blue 450-494nm (B)] and feeding regime (SF or RF, scheduled or random feedings, limited to white light and at 15ºC). The following experimental groups were established: 15_W_SF; 20_W_SF; 15_G_SF; 20_G_SF; 15_B_SF; 20_B_SF; 15_W_RF; 20_W_RF. During the experimental period, fish were reared under a 12D:12L photoperiod and daily fed at 3% of body weight. After 35 days, black-white preference tests were performed to assess anxiety-like behavior and blood samples were taken to measure cortisol. At the end of the experimental period (45 days), ten fish per treatment were euthanized with a lethal dose of MS-222 (400 mg/L) to collect hypothalamus and liver for RNAseq analysis focusing on genes related to stress, energy balance and photoreception. In addition, another group of fish for each treatment was sacrificed during a 24-hour period (every 4 hours) to sample blood, hypothalamus, pituitary and head kidney to measure cortisol and clock gene expression daily rhythms. Lastly, *ex vivo* skin explants from dorsal and ventral regions were examined to assess permeability to absolute irradiance (EI) with a spectroradiometer.

## Results and discussion

Temperature was the only condition that significantly increased plasma cortisol levels (Figs. 1A, 2A). This effect was not observed in fish reared under G or B lights, or under W light between SF or RF, possibly due to already elevated basal cortisol levels. Overall, fish exposed to 20ºC tend to exhibit longer latency to enter the aversive white area in the black-white preference tests and spend less time in it (Figs. 1B,C and 2B,C), suggesting increased anxiety under such conditions. Interestingly, at 20ºC, time spent in the aversive white area was diminished in fish under G compared to W; B presented intermediated values (Figs 1C). Remarkably, G light and RF condition did not highlight differences between 15 and 20ºC indicating already heightened anxiety. We confirmed the lights spectrum (Fig. 3A) and characterized skin permeability to absolute irradiance (EI) under each treatment. For the dorsal region, B light significantly increased EI permeability (Fig. 3B), whereas the ventral region showed reduced EI permeability (Fig. 3C).

In summary, our study shows that temperature is the primary factor significantly elevating plasma cortisol levels and anxiety-like behavior in rainbow trout, indicating heightened stress responses. Our findings underscore the complex interplay between environmental factors and fish welfare, highlighting the need for tailored aquaculture practices to optimize physiological and behavioral health in this species.

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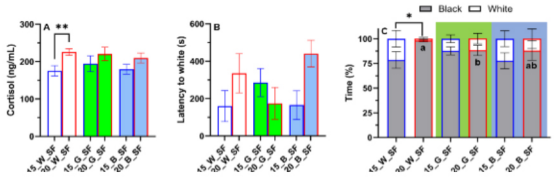

**Figure 1.** Effect of the temperature (15 or 20ºC) and light spectrum (W, G or B) on plasma cortisol (A), and on parameters measured in the black-white preference test (B-C). Data are represented as mean ± SEM (n=10). Different letters indicate significant differences for a given temperature (*1-way ANOVA)*. Asterisks for a given light condition between temperatures (*t-Student);* *p < 0.05; **p < 0.01.

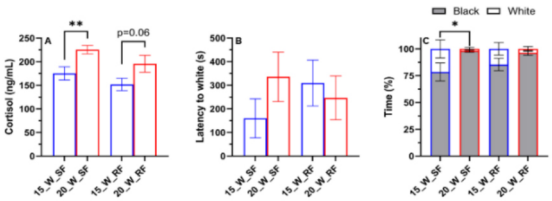

**Figure 2.** Effect of the temperature (15 or 20ºC) and feeding regime (SF or RF) on plasma cortisol (A), and on parameters measured in the black-white preference test (B-C). Further details in legend Figure 1.

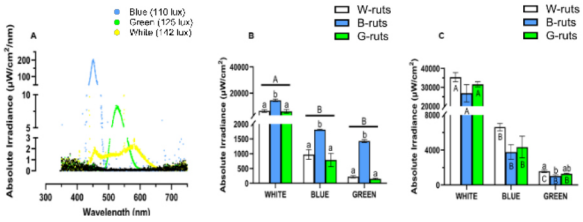

**Figure 3.** Light spectrums of used colors (A), and skin permeability to absolute irradiance in dorsal (B) and ventral regions (C) of trout exposed to different lights (x-axis) from W, B or G. Data are represented as mean ± SEM (n = 4-8). Different letters indicate significant differences. Lowercase: for a given tested light among individuals acclimated under W, B or G; capital letters: for a given acclimated group among different experimental lights (*2-way ANOVA)*; p < 0.05.

**Acknowledgements**

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# INITIAL INSIGHTS INTO GROWTH OPTIMIZATION IN AQUACULTURE OF JUVENILE SEA LAMPREY (*Petromyzon marinus*)

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## Introduction

Sea lamprey (*P. marinus*) populations across Europe—and particularly the Iberian Peninsula—are currently in sharp decline and classified as vulnerable due to overfishing, estuarine pollution, dam and canal construction, and riverbed gravel extraction, leading to local extirpations in many river systems. Today, lampreys are encountered only sporadically and seasonally (January–April), yet their cultural and gastronomic significance persists in Galicia (Spain), North Portugal and Bordeaux (France). Arbo's annual *Festa da Lamprea* drew over 35,000 visitors in 2023, while North Portugal's Penacova, Gondomar, and Entre-os-Rios host lamprey-themed weekends, and Bordeaux honors *lamproie à la bordelaise* with seasonal tastings—festivals that underscore both the lamprey's culinary appeal and its cultural importance, highlighting the urgency of conserving sea lamprey populations. The anadromous life cycle of *P. marinus* comprises a freshwater, filter-feeding larval stage; a parasitic juvenile stage—characterized by hematophagous micropredation—during downstream migration and marine residency; and a spawning adult phase upon return to rivers. We have a better understanding of the larval and adult stages in rivers, compared to the juvenile phase in the ocean, particularly its feeding physiology which remains understudied. In this pilot investigation, we evaluated the feasibility of captive rearing and developed a preliminary fattening protocol in which juveniles feed non-lethally on live Atlantic salmon (*Salmo salar*).

## Materials and methods

Juvenile sea lamprey (*P. marinus*; n = 35; 3.9 ± 0.8 g; 14.4 ± 0.9 cm) were intraperitoneally PIT tagged for individual tracking, then used in two independent feeding trials conducted May–August 2022. To benchmark against natural development, we maintained a fasting control group in fresh water (FW), mimicking the pre-feeding juveniles' stage. In Phase 1, lamprey were directly transferred to FW, brackish water (BW; 10–15‰), or SW (30‰) and held in single-tank replicates under each salinity for 30 days—each tank containing four Atlantic salmon (*S. salar*; 310.4 ± 38.7 g; 30.7 ± 1.1 cm) as live hosts, replaced whenever more than four lampreys attached to a fish or, if that threshold wasn't reached, weekly to prevent host mortality (replaced salmon recovered after exchange). In Phase 2, a separate cohort was held in two replicate tanks of BW for 39 days under the same four-salmon-per-tank and replacement protocol. Note that for all experiments, at least a 33% water exchange was performed weekly to maintain water quality, with additional exchange conducted whenever water clarity declined. Throughout both phases we tracked survival, attaching frequency (as a proxy for feeding behavior), and growth performance. At the end of Phase 1, all lamprey—including the FW fasting controls—were euthanized with a lethal dose of MS-222 (400 mg $L^{-1}$ buffered with $NaHCO_3$, pH 7) to collect hematocrit, plasma, gill, liver, caudal kidney, muscle, and proximal and distal intestine samples to characterize physiological status among groups. Additionally, liver and intestine morphometrics were recorded in relation to each fish's body mass.

## Results and discussion

In Phase 1 (Figs. 1A, B), salinity emerged as the key determinant of juvenile sea lamprey performance, with FW survival plummeting to 0% by day 20 due to complete mortality, whereas BW and SW sustained survival rates of 70% and 65%, respectively (Fig. 1A). Attachment to hosts commenced within the first 15 to 17 days across all salinities, achieving day 17 frequencies of ~10% in FW (prior to mass mortality); day 15 ~40% in BW, and days 15-17 ~50% in SW (Fig. 1B), underscoring the positive influence of elevated salinity on both survival and feeding initiation. Phase 2 (Figs. 1C, D) corroborated these trends: under BW, survival remained above 70% (Fig. 1C), while host-attachment frequency peaked at over 80% and feeding behaviour was evident from the experiment's outset (Fig. 1D).

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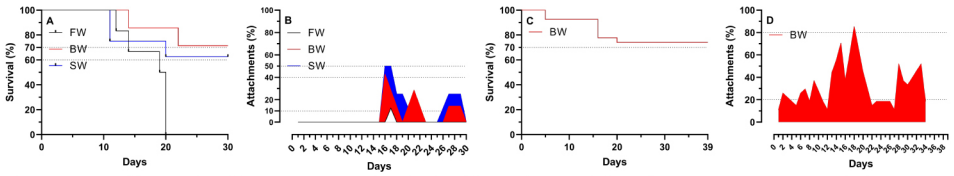


**Figure 1.** Effect of salinity on survival (A, C) and attaching frequency to host fish (B, D) on juvenile sea lamprey feeding trials. Figs.1A, B correspond to Phase 1, while Figs. 1C, D to Phase 2.

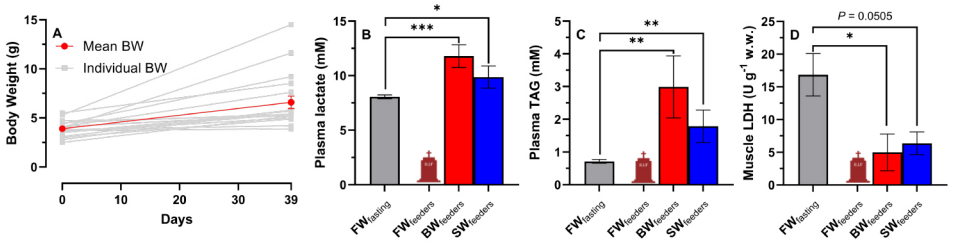


**Figure 2.** Growth performance in BW (A); plasma lactate (B), triglycerides (C), and muscle LDH (D). Fig.1A correspond to Phase 1, while Figs. 1B-D to Phase 2. ( ) indicates 100% mortality.

Post-feeding, juvenile lamprey showed high individual variability in growth performance but, overall, displayed substantial growth potential (on average, ~0.1 g per day; Fig. 2A), consistent with molecular data from the same individuals indicating active growth-related gene expression [1]. Plasma lactate levels rose significantly after feeding, mirroring the increase observed in plasma triglycerides (Figs. 2B, C). The surge in triglycerides indicates enhanced lipid uptake and storage following the exogenous nutrient supply. In contrast, muscle lactate dehydrogenase (LDH) activity declined markedly (Fig. 1D), likely reflecting a downregulation of glycolytic flux in muscle tissue as circulating nutrients became available.

In summary, our study indicates that salinity changes significantly enhance feeding and growth in juvenile sea lamprey. High survival and rapid host attachment occurred in individuals reared in BW and SW, while prolonged FW exposure led to mortality. This is the first comprehensive use of physiological analyses, supported by gut-content data, to confirm post-feeding status—showing strong growth, elevated plasma lactate and triglycerides, and reduced muscle LDH activity, signaling a shift to lipid metabolism. These results highlight the link between salinity, feeding, growth, and metabolism, and underscore the need for gentle handling, good water quality, and proper acclimation to maintain natural behaviors in captivity. Incorporating juvenile cultivation into full-cycle aquaculture could ease pressure on wild stocks, enable off-season supply and restoration efforts, and provide a consistent source for research—while ensuring host fish recover fully within a month.

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# EVALUATING THE SAFETY OF NATURE-BASED ANTIFOULING SOLUTIONS FOR SUSTAINABLE BIVALVE FARMING

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**Introduction**

Alongside with the growing global aquaculture, shellfish aquaculture has also been increasing over the last years, with oysters and clams representing more than half of the world's production [1]. Bivalve farming is usually carried out in cages or structures placed in natural environments, such as estuaries or other coastal areas. Despite the benefits of this type of aquaculture, including the ability to improve water quality and provide several ecosystem services, it faces a major challenge called biofouling [2]. Biofouling is the development of undesirable species, such as seaweeds and invertebrates, on the surface of submerged farm structures, or even on the surface of the cultivated shellfish, representing a large constrain to marine aquaculture by reducing water flow, competing for resources and, therefore, negatively impacting productivity. Biofouling management represents an economic cost of 5-10% in the marine aquaculture industry [3], however, its prevention is essential to achieve efficient productions. While strategies such as physical cleaning, biocidal coatings (some of them toxic to non-targeted organisms), and genetic selection are being employed [3], there is an urgent need for the development of more sustainable approaches, as well as comprehensive assessments of their ecological impacts.

This research group has been investigating natural antifouling alternatives, such as the use of bioactive compounds [4], some derived from cyanobacteria [5, 6]. The aim of the present study was to assess the impact of four nature-based potential antifouling products on a commercially relevant clam species, *Venerupis corrugata*.

**Methods**

*V. corrugata* juveniles were acclimated for 4 days and, after that, they were exposed to cyanobacteria bioactive compounds (portoamides; **PA**) [5], two nucleosides (hypoxanthine arabinoside (**HA**) and 2'-deoxyinosine (**DOI**) (unpublished data)), and a nature-inspired polyphenol (**GBA26**) [7] for 48h, at six different concentrations: 6.25; 12.5; 25; 50; 100; 200 µM. To perform the assay, 12-well plates were filled with filtered seawater (17ºC) and 5 clams per well and three wells per treatment were used. A negative (seawater) and positive ($CuSO_4$; 5 µM) controls were also added. Mortality was regularly checked and according to these preliminary results, the most suitable concentrations were chosen to perform a second trial. The tested concentrations were 6.25; 12.5 and 25 µM and six clams per well and five wells per treatment were used. After 48h of exposure, live clams were collected for oxidative stress analysis. Whole tissue was homogenized in pools of 5 clams and lipid peroxidation (LPO), total protein content and the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-*S*-transferase (GST) were assessed.

**Results and Discussion**

Results demonstrated that after 48h, **DOI** and **GBA26** induced no mortality across all tested concentrations (up to 200 µM). In contrast, **PA** showed a dose-dependent increase in mortality, reaching approximately 85-90% at 200 µM, indicating acute toxicity only at very high concentrations.

The evaluation of oxidative stress biomarkers provided further insights. Results demonstrated no significant differences in total protein content, CAT, or GST activities across treatments, suggesting that these compounds did not induce significant metabolic disruption or detoxification responses. However, a significant increase of SOD activity was observed with the highest tested concentration of **DOI** (25 µM) and in the presence of **GBA26** and **PA** (at all concentrations) ($p$-value $\leq 0.01$ to $\leq 0.0001$). This suggests that these compounds, particularly at higher concentrations, can induce an oxidative stress response in *V. corrugata* juveniles, promoting the partial activation of the first-line antioxidant defence mechanism. LPO levels showed no significant differences after treatments, suggesting that the compounds did not cause oxidative cellular membrane damage.

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These findings underscore the importance of thoroughly evaluating the environmental safety of antifouling compounds, particularly in relation to their effects on the target farmed shellfish species. Antifouling formulations should be carefully designed to minimize potential adverse impacts on aquaculture organisms such as *Venerupis corrugata*. Future long-term and field-based studies will be essential to confirm the suitability and ecological safety of these nature-based solutions for practical use in shellfish aquaculture.

**Acknowledgments**
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# LIFE CYCLE ASSESSMENT AND ENVIRONMENTAL RETURN ON INVESTMENT OF THE DIETARY SUPPLEMENTATION OF LYSO-PHOSPHOLIPIDS IN SHRIMP FEED

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## Introduction

Shrimp feed formulation requires increasing flexibility and cost-efficiency while minimizing environmental impact. Lyso-phospholipids (LPL) are functional additives that support this goal by enabling partial ingredient replacement without compromising nutritional performance. This study assessed the net environmental benefit of a lyso-phospholipid-based additive (Aqualyso) in shrimp feeds through Life Cycle Assessment (LCA) and Environmental Return on Investment (EROI) analyses.

## Material and methods

LCA was conducted in accordance with ISO 14040/44 standards (ISO, 2006a,b), the Product Environmental Footprint Category Rules (PEFCR) guidelines (European commission, 2021), and FAO LEAP recommendations (FAO, 2020). Biological performance data from controlled trials testing the additive with *Litopenaeus vannamei* were used to simulate commercial shrimp farming in Indonesia. System boundaries included feed ingredient sourcing, feed processing, transport, on-farm emissions, and energy consumption. Carbon footprint (CFP) was calculated at both the feed level and farm level, expressed as kg $CO_2$eq/kg feed or $CO_2$eq/kg liveweight of shrimp, respectively, as the functional unit. Additionally, the EROI was assessed as the ratio between the reduction in CFP at the farm level and the environmental impact associated with the production of the additive (Blonk *et al*., 2021).

## Results & conclusions

Across all tested scenarios, the inclusion of Aqualyso in reformulated feeds led to measurable CFP reductions at both feed and farm levels, while delivering a positive EROI. In Scenario 1, 0.75% soy lecithin was replaced with 0.1% Aqualyso , resulting in CPF reductions of 2% and 8% at feed and farm levels, respectively (Fig. 1A). The corresponding EROI was 66 kg $CO_2$eq saved per kg $CO_2$eq produced by the additive. In Scenario 2, 1% soy lecithin was replaced with 0.05% Aqualyso, resulting in CFP reductions of 5% and 14% at feed and farm levels, respectively, (Fig. 1B), and in an EROI of 234 kg $CO_2$eq. In Scenario 3, 0.75% of soy lecithin and 0.5% of fish oil were replaced with 0.1% Aqualyso, leading to 3% and 15% reductions in feed and farm CFP, respectively l (Fig. 1C), and to an EROI of 115 kg$CO_2$ eq.

In all scenarios, soybean meal remained the primary contributor to feed-related emissions (35-55%; Fig. 1). Aqualyso enabled reductions in soy lecithin content, thereby marginally lowering overall feed emissions. The observed CFP reductions at the farm level were driven by improvements in feed conversion ratio and growth rate. These findings demonstrate that Aqualyso contributes meaningfully to the sustainability of shrimp production through improved feed formulation flexibility and reduced CFP emissions.

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# A MARINE PROTEIN CONCENTRATE TO GAIN FLEXIBILITY IN THE FORMULATION OF FISH AND SHRIMP FEEDS

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## Introduction

The development of sustainable and efficient feed additives is a major priority in aquaculture. Marine peptide concentrates, derived from high-quality marine ingredients, have emerged as promising ingredients due to their digestibility, balanced amino acid profiles, and potential functional benefits. However, species-specific responses and optimal inclusion levels must be carefully evaluated. This review summarises results from four feeding trials in three commercially important species, exploring the use of a marine peptide concentrate (Pepsea F) across different inclusion levels and diet formulations. Particular attention is given to the functional role of the additive in enhancing growth and feed efficiency under varying nutritional contexts.

## Material and methods

Four independent trials were conducted under controlled conditions using sea bream (*Sparus aurata*), catfish (*Clarias sp*.), and shrimp (*Litopenaeus vannamei*). Experimental diets were formulated with different fish meal (FM) levels (7%–20%), reflecting both reduced and moderate FM strategies. Pepsea F was incorporated at inclusion levels ranging from 0.4% to 2.6%, while the product was not used in control feeds. Husbandry and feeding protocols differed by species and life stage. Growth performance parameters including feed intake, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and survival were recorded and statistically analysed ($p < 0.05$).

## Results & conclusions

In Trial 1, sea bream fed a 10% FM feed supplemented with 2.6% Pepsea F for 10 weeks showed a significant 10% increase in feed intake, 12% improvement in growth, and 17% improvement in FCR relative to the control group.

In Trial 2, sea bream fed a 20%FM feed supplemented with 0.6% Pepsea F showed a significant 3% increase in feed intake and 4% improvement in growth after 11 weeks and compared to the control feed.

In Trial 3, catfish fed a feed containing 8% FM and 0.4% Pepsea F achieved a significant 10% higher feed intake, 3% greater growth performance, and 5% better FCR (not significant) after 8 weeks and in relation to the control feed.

In Trial 4, shrimp fed a 7% FM feed and supplemented with 0.5% Pepsea F over 7 weeks exhibited a significant 21% increase in feed intake and a numerically 3% improvement in growth in relation to the control group.

Overall, Pepsea F at both low (0.4 – 0.6%) and higher (2.6%) inclusion levels showed efficacy to improve the performance quality of a range of feed formulations and in different species. These trials demonstrate that Pepsea F can function as both palatability enhancer and performance booster and stands as an alternative to gain flexibility in formulation without compromising productivity.

# MORE THAN A PROTEIN SOURCE: MULTIFUNCTIONAL BENEFITS OF METHANOTROPH-DERIVED PROTEIN (FEEDKIND) IN FEED PROCESSING AND FISH HEALTH

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## Introduction

The rising demand for aquaculture production has intensified reliance on high-quality protein ingredients, particularly fish meal (FM), which remain nutritionally valuable but increasingly constrained by price volatility, limited supply, and ecological concerns. Alternative proteins from terrestrial animals and plants offer partial substitutions but often introduce variability in nutrient composition, anti-nutritional factors, or processing challenges that compromise feed efficiency and fish performance. Against this backdrop, methanotroph bacteria protein (MBP) produced from *Methylococcus capsulatus* through natural-gas fermentation, has emerged as a consistent and sustainable alternative. MBP is characterized by high protein content, favorable amino acid profile, excellent digestibility, and functional properties that influence both extrusion performance and fish physiology. Importantly, its production requires minimal agricultural land or water use, thereby reducing the environmental footprint compared with other alternative protein sources. Beyond replacing FM, MBP offers unique potential to improve feed processing efficiency and fish health condition at relatively low inclusion levels. This work synthesizes findings from three studies that assess the efficacy of the MBP-based FeedKind (FK, Adisseo) in improving feed processing efficiency and promoting fish health in commercial fish species.

## Material and methods

Th first study evaluated the applications of FK for feed production to improve the physical qualities of low-starch extrudates (Liu *et al*., 2023). The second study evaluated FK supplementation on growth performance and gut health of spotted seabass (*Lateolabrax maculatus;* Zhang *et al*., 2023). The third study investigated the effect of FK in largemouth bass ((*Micropterus salmoides*) to enhance antioxidant capacity and modulate the gut microbiome (Guo *et al.,* 2022).

## Results and discussion

The extrusion study demonstrated that the inclusion of 6% FK in 40% FM feeds reduced oil leakage by 35%, improved pellet expansion and durability by 4%. FK inclusion also lowered the required moisture level around 10% for conditioning, and 11% decrease in the temperature required in extrusion, these together reduced overall energy consumption of 37 kWh per ton of feed produced compared to the control feed. These conditions reduced drying time, water and energy use, contributing to more sustainable production of extruded feeds without compromising pellet quality.

Feeding trials further confirmed the functional health benefits of FK at relatively low inclusion levels. In Spotted seabass (3 g initial weight; 8 weeks duration), supplementation of 2% FK in high soybean meal content feeds (32% SBM, 28% FM) had a predominant role on cytokines regulation in the gut, alleviating soybean meal-induced enteritis. In largemouth bass (48 g initial weight; 10 weeks duration), 3-6% FK supplementation improved liver function as denoted by a decrease in plasma alanine aminotransferase concentration and improved antioxidant capacity in plasma and liver marked by a 32% and 30% increase of superoxide dismutase and by a 38% and 81% decrease of malondialdehyde concentration, respectively. Lastly, an increase in the beneficial microbial richness and diversity in the gut microbiome was also found in those groups fed FK.

## Conclusion

Collectively, these studies demonstrate that MBP-based Feedkind delivers consistent benefits beyond protein replacement. At low inclusion levels (2 – 6%), FK improves feed processing efficiency and promotes physiological resilience and gut health across fish species. These outcomes position FK as a multifunctional, sustainable protein source that can enhance both feed production efficiency and fish health in aquaculture.

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# WINDING THE CLOCK – EXPLORATION OF THE EARLY DEVELOPMENT OF THE CIRCADIAN CLOCK MECHANISM IN EURASIAN PERCH

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## Introduction

For over 100 million years, the stable day-night cycle has driven the evolution of circadian clocks across different organisms, allowing them to anticipate and adapt to daily and seasonal environmental changes. In finfish, the circadian system regulates essential processes like digestion, cell proliferation, stress responses, and reproduction (Vallone et al., 2007). On the molecular level, the circadian clock genes (*clock, arntl, per, cry*) form the foundation of cellular time perception by generating rhythmicity through positive and negative transcription-translation feedback loops. While studies in typical model organisms, especially zebrafish, have shown early onset of circadian gene expression (Dekens & Whitmore, 2008), much less is known about this process in temperate commercial species such as Eurasian perch, a freshwater fish of significant ecological and aquaculture importance. Understanding when and how this mechanism becomes functional during early development is crucial, as it may enable adjustment of hatchery protocols for domesticated stocks (Cahill, 2002). This study aims to explore the development of the circadian clock in early life stages (embryos and larvae) of Eurasian perch, using high-resolution sampling. Relatively long period of embryogenesis and larval metamorphosis in Eurasian perch compared to species like zebrafish provided us with a unique opportunity to gain insights into circadian regulation of closely spaced developmental stages. Our objectives were to: (1) map the expression of core circadian genes at key developmental stages, (2) determine the onset of rhythmicity and compare it with other investigated species, and (3) explore the biological and aquaculture implications of our findings.

## Materials and methods

Eight families of Eurasian perch were created by controlled reproduction. Eggs were fertilized in vitro using cryopreserved semen (Judycka et al., 2022), and incubated in a recirculating aquaculture system (RAS) under a 14L:10D photoperiod, which simulates the natural light conditions during the spawning and early developmental stages of this species. The hatched larvae were reared in the same light conditions as embryos, until 36 days post hatching (DPH) following the standardized procedure (Palińska-Żarska et al., 2020).

Embryos and larvae were sampled continuously, every 4 hours, from the moment of fertilization to 3 DPH (11 days). From 3 DPH, larvae were sampled every 4 days, also in 4-hour intervals. The RNA from embryos and whole-larvae was extracted and analyzed for circadian clock gene expression (*clockb, cry1b, cry2, per1b, arntl1a*) using real-time qPCR. Rhythmicity of gene expression was analysed using Cosinor (Molcan, 2023). Gene expression correlations were assessed using Spearman's correlation matrices in RStudio.

## Results

During embryonic development, expression patterns of clock genes were random with no clear daily rhythm, indicating the circadian clock mechanism had not yet become functional. The Eurasian perch larvae displayed rhythmic expression of circadian genes starting at 3 DPH, coinciding with onset of exogenous feeding, with peaks and troughs in *per1b*, *clockb*, *arnlt1a*, and *cry1b* aligned with transitions between the day and night. The highest expression amplitude was recorded for *cry1b*, while *arnlt1a* had the lowest. Rhythmicity of the analysed genes persisted and became more consistent at the end of the larval period (around 28 DPH).

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**Discussion**

This study provides the first detailed description of the development of circadian clock gene rhythmicity in Eurasian perch. The delayed onset of rhythmicity in Eurasian perch in comparison with other investigated teleosts (e.g., medaka, zebrafish, and Senegalese sole) may be linked to its slower development. This suggests that circadian clock development could be tied to the specific ecological needs and life history traits of each species. Prolonged embryogenesis of the Eurasian perch which takes place in a relatively safe microenvironment of the chorion, further protected by gelatinous matrix around the eggs might delay the need for a functional circadian clock. Therefore, the Eurasian perch might prioritize the development of other functions, such as visual and motor systems, before activating the circadian mechanism. The rhythmic expression of circadian clock genes began synchronously at 3 DPH. The timing of the onset of circadian clock gene rhythmicity coincides with the beginning of exogenous feeding. The presence of a functional circadian clock at this developmental stage may optimize feeding strategies by synchronizing larval activity with diel zooplankton migrations, thereby enhancing foraging efficiency and reducing predation risk. (Mikheev & Wanzenböck, 2010). As development progressed, these rhythms became more consistent in juveniles, likely reflecting the maturation of the nervous system and improved regulation of circadian processes.

This study not only enhances our understanding of circadian clock development in non-model species but also contributes to the broader knowledge of how circadian rhythms evolve in response to environmental factors. Our data shed light on how early life stages in this species are synchronized with natural light-dark cycles, which is critical for survival, growth, and feeding in the wild. In an ecological context, disruptions to these rhythms - such as through increasing levels of artificial light at night (ALAN) - could interfere with key biological processes, potentially affecting recruitment success and population dynamics. From an aquaculture perspective, the common practice of rearing Eurasian perch larvae under constant light conditions could profoundly alter the natural development of their circadian system, with potential consequences for growth, digestion, behaviour, stress resilience, and long-term welfare.

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# EFFECTS OF DIETARY *Artemisia annua* SUPLEMENTATION ON OXIDATIVE STATUS AND PLASMA PARAMETERS OF *Sparus aurata*

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## Introduction

European strategies in aquaculture are prioritising sustainable feeding practices and the use of functional additives to reduce dependence on antimicrobials. Medicinal plants and plant-derived compounds represent promising alternatives to antibiotics and immunoprophylactics, offering environmental safety and cost-effectiveness compared to synthetic drugs. Among these, *Artemisia annua* has gained attention as a functional feed additive. This fast-growing annual herb contains artemisinin and other bioactive compounds with diverse pharmacological properties, including antibacterial activity and modulation of pathogen-induced immune and metabolic responses (Wang et al., 2023). Artemisinin generates reactive oxygen species that damage parasite cell membranes and disrupt mitochondrial function (Li et al., 2005). Given these bioactivities, dietary *A. annua* may enhance fish health, strengthen disease resistance, and reduce antibiotic reliance in aquaculture. The present study evaluates the effects of a diet supplemented with *A. annua* leaves on oxidative stress markers and plasma biochemical parameters in gilthead sea bream (*Sparus aurata*).

## Material and methods

For the present study, a total of 144 juvenile sea bream (47.18 ± 6.60 g) were randomly distributed into 12 polyethylene tanks (1 $m^3$ each) at the Centro Oceanográfico de Canarias (COC-IEO), CSIC. In each tank, six individuals were randomly selected and tagged with microchips. Four treatments (2 diets x 2 injections) were evaluated, with 3 tanks per treatment. Two dietary treatments were tested: a control group received a commercial diet (control; 6 tanks) and an experimental group that received the same diet plus 30 g/kg of dried *A. annua* powdered leaves (experimental; 6 tanks). Fish were fed daily at 3 - 5% of their biomass, divided into three equal meals. Following 37-days, three fish per tank were intraperitoneally injected with either 5 mg/kg body weight of *Escherichia coli* lipopolysaccharide (LPS; 3 tanks per diet) or an equivalent volume of phosphate-buffered saline (PBS; 3 tanks per diet). Forty-eight hours later, injected fish were euthanised and sampled. Subsequently, the *A. annua* dose in the experimental diet was reduced to 10 g/kg and the same sampling procedure repeated after 62-days. At both times, blood was collected from the caudal vein using heparinised syringes, and gills, liver, intestinal and muscular samples were obtained and stored at −80 °C until biochemical and antioxidant enzyme response analyses were performed.

## Results

At 37 days, glucose levels decreased in LPS-injected fish fed the control diet ($p = 0.033$), while chloride and lactate remained unaffected by diet or LPS. At 62-days, *A. annua* supplementation significantly reduced glucose compared to control dietary and LPS groups ($p = 0.001$ in control, $p = 0.000$ in LPS), but increased lactate ($p = 0.022$ in control, $p = 0.021$ in LPS). At both times, an ionic imbalance was observed: potassium increased ($p = 0.000$ at 37-days and 62-days), while sodium decreased ($p = 0.000$ at 37-days; $p = 0.004$ at 62-days) with *A. annua* supplementation compared with the control diet. *A. annua* also altered antioxidant enzymes and lipid peroxidation. At 37-days, *A. annua* feeding reduced intestinal catalase (CAT) activity after LPS injection ($p = 0.011$). In the liver, superoxide-dismutase (SOD) activity showed variations associated with both diet and LPS treatment, while in muscle, glutation-S-tranferase (GST) activity decreased with *A. annua* supplementation (Table I). Regarding lipid peroxidation, malondialdehyde (MDA) content in the intestine and gills decreased after LPS injection with *A. annua* diet ($p = 0.024$ and $p = 0.046$, respectively). At 62-days, gills-CAT and GST decreased by *A. annua* feeding (CAT: $p = 0.000$; GST: $p = 0.000$). Moreover, GST increased after LPS injection in *A. annua* diet. In muscle, CAT activity varied with diet and LPS injection, while in the liver, *A. annua* reduced SOD and increased MDA in both groups (Table I). In the intestine, MDA content increased with the experimental diet following LPS injection ($p = 0.000$).

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Table I. Mean ± SEM. Catalase (CAT), superoxide-dismutase (SOD), glutation-S-tranferase (GST) (U $min^{-1}$ $mg^{-1}$ protein), and malondialdehyde content (MDA, nmol MDA $mg^{-1}$ protein) in liver and muscle of *S. aurata* under the experimental treatments at both sampling times.

| | | | Control | *A. annua* | LPS | | ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Control | *A. annua* | D | I | In |
| **Liver** | 37-days | CAT | 259.6 ± 12.8 | 263.0 ± 28.3 | 227.6 ± 8.8 | 324.8 ± 19.4 | | * | |
| | | SOD | 1.4 ± 0.1 | 2.1 ± 0.3 | 1.7 ± 0.1 | 1.5 ± 0.4 | * | * | * |
| | | GST | 119.7 ± 5.5 | 122.0 ± 12.0 | 119.7 ± 8.4 | 112.9 ± 9.8 | | | |
| | | MDA | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0.1 | 0.7 ± 0.1 | | | |
| | 62-days | CAT | 224.9 ± 9.6 | 219.5 ± 7.3 | 238.1 ± 16.9 | 246.0 ± 11.4 | | | |
| | | SOD | 1.5 ± 0.1 | 0.8 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.2 | * | | * |
| | | GST | 122.2 ± 5.2 | 115.9 ± 5.3 | 131.9 ± 8.1 | 131.5 ± 7.2 | | * | |
| | | MDA | 0.6 ± 0.0 | 0.9 ± 0.0 | 0.5 ± 0.0 | 0.8 ± 0.1 | * | * | * |
| **Muscle** | 37-days | CAT | 2.5 ± 0.3 | 3.0 ± 0.3 | 2.8 ± 0.3 | 3.2 ± 0.5 | | | |
| | | SOD | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.0 | 0.5 ± 0.0 | | | * |
| | | GST | 4.0 ± 0.1 | 3.5 ± 0.1 | 3.9 ± 0.1 | 3.8 ± 0.1 | * | | |
| | | MDA | 0.6 ± 0.1 | 0.4 ± 0.0 | 0.8 ± 0.2 | 0.7 ± 0.1 | | | |
| | 62-days | CAT | 1.0 ± 0.1 | 0.9 ± 0.1 | 1.5 ± 0.1 | 1.0 ± 0.0 | * | * | |
| | | SOD | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | | | |
| | | GST | 4.3 ± 0.4 | 3.4 ± 0.2 | 4.3 ± 0.4 | 4.0 ± 0.3 | | | |
| | | MDA | 1.1 ± 0.2 | 0.8 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | * | | |

D, diet; I, LPS-injection; In, interaction. * Significant differences (Two-way nested ANOVA).

Discussion and conclusions

Despite previous studies have demonstrated the efficacy of *A. annua* supplementation in improving health outcomes, these effects were not observed in the present study. Decreases in glucose levels have also been reported by Eisenman et al. (2011) with *A. draconunlus* supplementation in rainbow trout, likely due to its antidiabetic properties. The observed increase in lactate, indicative of metabolic stress or anaerobiosis, likely results from disrupted ionic homeostasis, suggesting a negative physiological response to *A. annua* supplementation. *A. annua* has been shown to reduce lipid oxidation and regulate lipid metabolism in *Hexagrammos otakii* (Wang et al., 2023) and *Cyprinus carpio.* (Hoseini et al., 2022). Nevertheless, in this study causes clear imbalances in oxidative response enzymes and differences in MDA content. Previous research using *A. arborescens* as a dietary supplement has demonstrated detrimental effects on growth performance, immune response, and blood parameters in sea bream (Tzortzatos et al., 2024), corroborating our present findings. Consequently, supplementation with *A. annua* appears to induce stress in sea bream, highlighting the need for further research on lower dosages to assess its potential benefits.

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# EFFECTS OF MS-222 ANAESTHESIA ON OXIDATIVE STRESS IN *Seriola dumerili*

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Introduction

*Seriola dumerili* is a high-value species with significant potential for aquaculture diversification, a rapidly growing industry that faces challenges such as developing appropriate procedures and conditions to ensure animal welfare and optimal growth. Anesthesia plays a key role in minimizing handling stress and preventing physiological alterations during experimental or farming procedures. Once absorbed through the gills, anesthetics reach the central nervous system, reducing stress responses by lowering neural activity, metabolism, and oxygen consumption (Zahl et al., 2012). The ideal anaesthetic should act rapidly, allow quick recovery, and avoid disrupting physiological balance. However, most anesthetics can act as stressors, altering respiration, circulation, metabolism, and oxidative status. Elevated levels of reactive oxygen species (ROS) and changes in key antioxidant enzymes, such as catalase (CAT), glutathione S-transferase (GST), and superoxide dismutase (SOD), are indicators of oxidative stress and tissue damage. Tricaine methane-sulfonate (MS-222) is one of the most commonly used anesthetics for poikilotherms worldwide. Its efficacy is influenced by both environmental factors and biological characteristics, including age, sex, size, weight, lipid content, species, and fish density. Consequently, variations among species, developmental stages and social behaviour should be considered when determining the appropriate dosage (Topic Popovic et al., 2012). Due to limited knowledge on the physiological effects of anesthesia in *S. dumerili*, establishing species-specific protocols to monitor and mitigate stress remains a critical challenge. This study aimed to assess the impact of MS-222 on oxidative damage and antioxidant responses in *S. dumerili*, with the objective of identifying potential stress biomarkers for this species.

Material y methods

For this study, a group of 16 captive-born greater amberjack (*Seriola dumerili*) (3.9 ± 1.0 kg; 55.3 ± 5.1 cm) from the Centro Oceanográfico de Canarias (COC-IEO, CSIC; 28°29'56.1"N, 16°11'44.2"W) were anesthetized with MS-222 (tricaine methanesulfonate) at a concentration of 0.1 g/L of seawater, achieving complete loss of reflexes in under 3 minutes. Four groups were established: a control euthanised after initial anaesthesia, and three experimental groups differing in recovery time before a second anaesthesia and sacrifice: 0 h, 1 h, and 24 h. For each group, the times of loss of balance and loss of reflex activity were recorded, followed by the recovery time of the anesthetized fish. After euthanasia, gills, brain, liver and muscle tissues were collected and stored at –80 °C for subsequent analysis of antioxidant enzyme activity.

Results

MS222 had pronounced effects on oxidative stress markers across tissues. CAT activity in the brain was significantly lower than the control at all sampling times (0 h: $p = 0.003$; 1 h: $p = 0.001$; 24 h: $p = 0.011$). In the liver, CAT activity was highest at 0 h, but not significantly compared to the control (Fig. 1E). SOD activity showed a significant decrease in the gills compared to the control at both 0 h and 24 h (Fig. 1C). In muscle, SOD activity differed significantly among experimental times, with the highest value at 0 h; however, no significant differences from the control were detected, and either in the liver. GST activity in the liver differed significantly from the control only at 24 h (Fig. 1F). In muscle, GST activity varied significantly among times, with the lowest value at 24 h; however, the only significant difference from the control occurred at 0 h ($p = 0.012$). MDA content in the gills was significantly reduced compared to the control, with the greatest decrease at 0 h (Fig. 1D), whereas no significant differences were detected in the liver (Fig. 1H).

Discussion and conclusions

The sedation and anaesthesia times observed with MS-222 in *S. dumerili* were adequate and comparable to those reported by Maricchiolo and Genovese (2011). However, MS-222-induced oxidative disturbances were found to be tissue-specific. Several studies have shown that MS-222 alters fish physiology and blood parameters (Topic Popovic et al., 2012). Oxidative stress in fish involves increased oxidation products and changes in the activities and concentrations of the components of the endogenous antioxidant system, with oxidative stress being a primary mechanism of tissue damage and physiological disorders. Sustained

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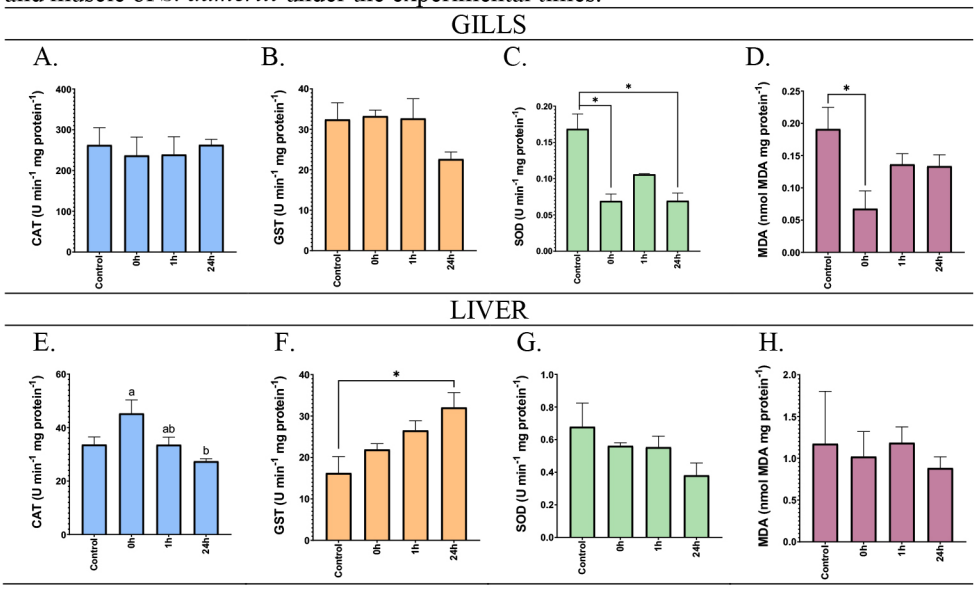
Fig.1. Catalase (CAT), superoxide-dismutase (SOD), glutation-S-tranferase (GST) (U $min^{-1}$ $mg^{-1}$ protein), and malondialdehyde content (MDA, nmol MDA $mg^{-1}$ protein) in gills and muscle of *S. dumerili* under the experimental times.




inhibition of CAT in the brain and a significant reduction in SOD in the gills indicate a deterioration of antioxidant defences in two critical systems. The gills are the main site of gas and ion exchange, while the brain is the centre of neural regulation; thus, increased reactive oxygen species in these tissues can have serious implications for fish behaviour and physiology. These findings suggest that, while MS-222 provides rapid anaesthesia, it significantly alters the oxidative balance in key organs in *S. dumerili*, making it less suitable for contexts where oxidative homeostasis is critical, especially in studies related to neural or gills physiology.

Acknowledgements

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# PREVENTLICE: A DECISION SUPPORT TOOL FOR SEA LOUSE PREVENTION BASED ON HYDRODYNAMIC AND SITE EXPOSURE DATA

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## Introduction

In regions where salmon lice (*Lepeophtheirus salmonis*) are endemic, Atlantic salmon (*Salmo salar*) farmed in open sea-cages accumulate lice, and eventually must be harvested or deloused to remain under mandated thresholds. This brings substantial animal welfare and economic costs. As most infective lice larvae are found near the surface, new infestations can be prevented or slowed by installing mesh or tarpaulin barriers at relevant depths to keep planktonic louse larvae out of the cage, by encouraging fish to swim deeper through use of deep lights and feed delivery, or by submerging the entire cage to hold fish below the depths that usually contain the most infective larvae. However, each approach has benefits and drawbacks depending on local hydrodynamic conditions and other factors. Within the FHF project PreventLice, we compiled new and existing knowledge on how salinity, temperature, density, currents, and wave exposure affect the efficacy of barriers and deep lighting/feeding methods, and by combining this knowledge with local predictions from a national-scale hydrodynamic model, generated bespoke initial recommendations for every active salmon farm in Norway, available through a free web app and data archive. The app is now being expanded to address the use of submerged cages, develop a universal index of wave exposure, and provide decision support for new sites as part of new FHF projects BetterWel, SafeSubmergence, and LusePraksis.

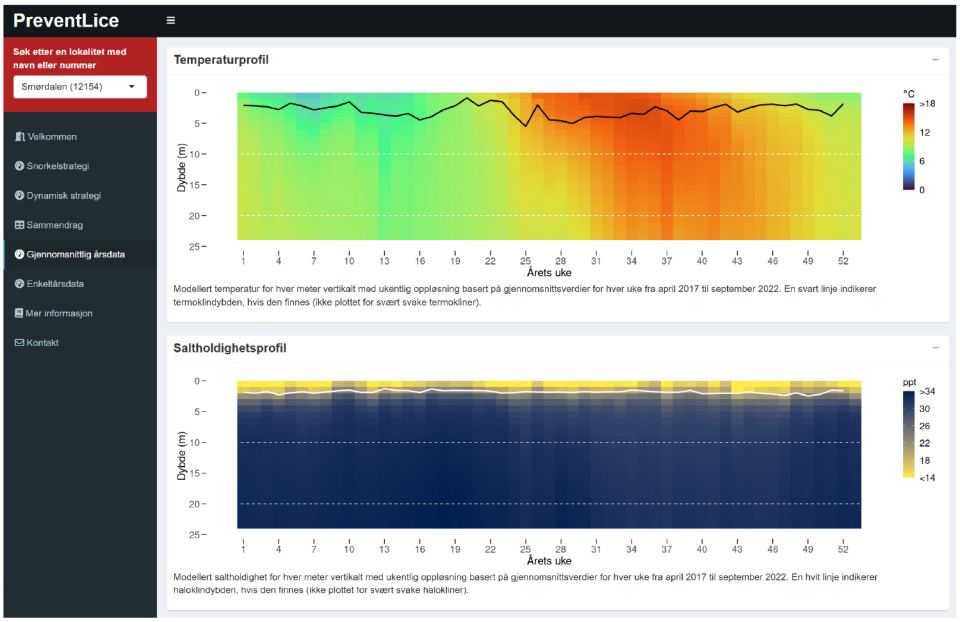


**Figure 1**. Screengrab from the freely accessible PreventLice web app, available in Norwegian at: https://havforskningsinstituttet.shinyapps.io/preventlice/

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**Methods**

Predictors of successful use of louse barriers were identified by combining existing knowledge (meta-analysis) with new data collection targeted at identified knowledge gaps. This was done through 60 sampling visits at commercial farms distributed along the Norwegian coast throughout all seasons. For over 1000 active salmon farming sites in Norway, we then extracted hydrodynamic and infestation pressure hindcasts from the Regional Ocean Modeling System models NorFjords160 and NorKyst800, and made site-specific recommendations based on the alignment between modelled site conditions and thresholds for efficacy identified through meta-analysis and new data collection. The site-specific hydrodynamic data and recommendations were made available by developing a web app using the *shiny* package for R (Figure 1). We are now processing new data on efficacy of 'skirt' barriers and submerged cages at collaborating sites, which will be used to update recommendations for use of these approaches, especially with respect to site exposure indices.

**Results**

'Snorkel' barriers reduced louse infestations by 80% or more in ideal conditions, when salinity is uniform throughout the water column, but efficacy was significantly reduced when conditions deviate from ideal. Even in ideal conditions, snorkel cages reduced growth compared to controls and lead to increased amoebic gill disease risk, and are increasingly unpopular due to these and other challenges. In contrast, dynamically deployed 'skirt' barriers in combination with behavior modification reduced infestations by >50% compared to standard production cages, regardless of temperature and salinity variability and without negatively impacting growth, gill health, or other welfare metrics. Efficacy of both approaches was reduced at sites with exposure to large waves, but not strongly impacted by surface current speeds of up to 0.8 m $s^{-1}$. Further results will be available by the time of the conference.

# TOWARDS ON-SITE DIAGNOSTICS: A LAMP-BASED APPROACH FOR THE RAPID DETECTION OF *Vibrio harveyi* IN AQUACULTURE

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## Introduction

*Vibrio harveyi* (*Vh*) is a Gram-negative halophilic bacterium found in marine environments worldwide that can cause disease (vibriosis) in numerous aquatic animals, including cultured species. Nowadays, epizootics and outbreaks caused by *Vh* are increasing due to its wider distribution because of global warming (Amaro et al*.,* 2020). Early and accurate diagnosis is crucial for *Vh*-vibriosis management, but traditional methods do not cover these requirements. PCR-based approaches offer improved specificity, but they usually require expensive specialized equipment. However, loop-mediated isothermal amplification (LAMP) presents an insightful alternative that allows for a rapid and highly specific DNA amplification i*n situ* at isothermal temperatures, making it suitable for field deployment. Previous studies on LAMP assays for *Vh* have focused on the *toxR* and *dnaJ* genes, but they have shown limitations (Rahman et al., 2022). In contrast, the *vhhP2* gene, encoding a putative outer membrane protein, has emerged as a promising, highly conserved target across the species. The present study describes the development of a new LAMP assay, targeting the *vhhP2* gene, for *Vh* detection in aquaculture settings.

## Materials and methods

Bacterial strains: a collection of *Vh* strains, representatives of the dominant serotypes (Amaro et al. 2020), was used. Clinical and environmentally relevant *Vibrio* species were also included: *V. vulnificus, V. cholerae, V. parahaemolyticus, V. alginolyticus, V. rotiferianus, V. campbellii* and *V. anguillarum*. Strains were grown on Tryticasein Soy Agar or Luria-Bertani broth supplemented with 1 % NaCl (TSA-1 or LB-1) at 28 ºC.

DNA extraction: genomic DNA from bacterial strains was extracted using a modified boiling method. After a final centrifugation, the supernatant containing the extracted DNA was collected and stored at -20 ºC for subsequent use.

**Table 1.** Set of LAMP primers targeting the *vhhP2* gene of *Vibrio harveyi.*

| LAMP primer | Size (bp) | Sequence (5'-3') |
|---|---|---|
| vhhP2_F3 | 24 | TTACCCTACTAGAACTTATCATTG |
| vhhP2_B3 | 19 | TTCCTGTGTTCCACCATTG |
| vhhP2_FIP | 38 | ACGCATCGTCTTGGAGGTTTATTCTCGGCATCCTAGCT |
| vhhP2_BIP | 43 | TCAAGCAAAAATGGAATCCATCGCATTGGCTTTGGGTAAATCG |
| vhhP2_LF | 20 | AAAACGGGGAGCTGCAACAA |
| vhhP2_LB | 20 | TGACCAATTCGAAACAGGCG |

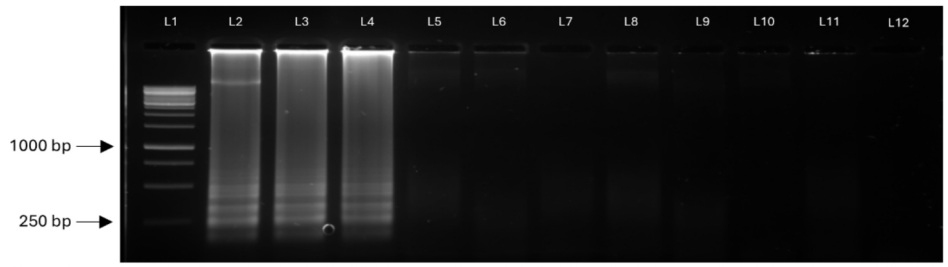


**Figure 1. Gel electrophoresis of the LAMP assay**. Lane (L) 1: 1 kb DNA ladder; L2: *Vibrio harveyi* (*Vh*) C2; L3: *Vh* C3; L4: *Vh* 203; L5: *V. vulnificus* CECT 4999; L6: *V. cholerae* B4; L7: *V. alginolyticus* Lub 1; L8: *V. anguillarum* Lab1; L9: *V. parahaemolyticus* TW2; L10: *V. campbellii* NCIMB 1894$^{T}$; L11: *V. rotiferianus* CAIM 577$^{T}$; L12: negative control (distilled water).

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Primer design: a set of six LAMP primers, comprising two inner (FIP/BIP), two outer (F3/B3), and two loop (LF/LB) primers, were specifically designed to target the conserved *vhhP2* gene region of *Vh* using GLAPD software (Jia et al., 2019). To ensure specificity and prevent non-specific hybridization, all primer sequences were rigorously verified *in silico* using Primer-BLAST tool. The final selected primers were synthesized by Integrated DNA Technologies (IDT, Belgium).

LAMP amplifications: LAMP reactions were performed in a total volume of 25 µL. Each reaction mixture contained: 1.6 µM each of FIP and BIP, 0.2 µM each of F3 and B3, 0.4 µM each of LF and LB, 1.4 mM each deoxynucleoside triphosphate (dNTPs), 8 mM $MgSO_4$, 1 µL of target DNA and 8 U of Bst 2.0 WarmStart ® DNA Polymerase (New England Biolabs). Amplifications were carried out at a constant temperature of 65 °C for 15 min, followed by enzyme inactivation at 80 °C for 10 min. Specificity was evaluated using DNA templates from the selected *Vibrio* species. Sensitivity was assessed by testing 10-fold serial dilutions of *Vh* genomic DNA. LAMP products were subsequently analyzed by 1.8 % agarose gel electrophoresis for visualization.

**Results and Discussion**

The developed LAMP assay, targeting the *vhhp2* gene with a comprehensive set of six primers (**Table 1**), demonstrated excellent results for *V. harveyi* detection.

Specificity assessment. All *Vh* strains tested in the study yielded a positive amplification signal. Crucially, the assay exhibited no cross-reactivity with any of the *Vibrio* species tested (**Figure 1**). Its high specificity is a powerful advantage, particularly in complex aquatic environments where multiple *Vibrio* species coexist, as occurs in aquaculture settings.

Sensitivity evaluation. The LAMP assay achieved a limit of detection (LOD) of 100 fg/µL of *Vh* genomic DNA. Its sensitivity was higher than most conventional PCR-based methods, even within a rapid 15-minute amplification period. Thus, the potential of this technique for early *Vh* detection is highlighted, even in the initial stages of infection or in environmental samples where bacterial load might be minimal.

**Conclusions**

We have developed a novel LAMP assay for *V. harveyi* detection, targeting the conserved *vhhP2* gene, highly sensitive and specific.

This rapid, isothermal diagnostic tool offers a cost-effective and practical solution for on-site application in aquaculture.

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# MOLECULAR WEIGHT OF DIETARY PROTEIN HYDROLYSATES IMPACT SOLE METAMORPHOSIS AND THERMAL CHALLENGE

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**Introduction**
Senegalese sole (*Solea senegalensis*) is a species of high commercial value in southern Europe, particularly in Portugal and Spain. The development of microdiets for flatfish larvae is essential to optimize hatchery protocols and augment juvenile quality. During early developmental stages, marine fish larvae have a limited ability to digest and absorb complex proteins (Engrola et al., 2009; Gamboa-Delgado et al., 2008). Therefore, including protein hydrolysates in larval feed formulations known to enhance protein absorption and growth in several marine fish species may be a viable nutritional strategy to overcome this biological constraint (Canada et al., 2017; Delcroix et al., 2015; Srichanun et al., 2014). Protein hydrolysates present a high bioactive potential for marine larval production, particularly by modulating antioxidant defences, enhancing immune responses, and improving digestive function. Their bioactive peptides can help maintain redox balance, support mucosal immunity, and stimulate enzyme activity, contributing to healthier and more resilient animals (Canada et al., 2017; Delcroix et al., 2015). The aim of the present study was to investigate the potential of incorporating protein hydrolysates in microdiets to improve larval quality in Senegalese sole, focusing on growth and resilience.

**Materials and Methods**
The trial was conducted at CCMAR's experimental facilities. Larval rearing took place in 100L tanks in RAS under optimal abiotic conditions for the species. Larvae were co-fed with microdiets and live-prey from mouth opening (2 days after hatching – DAH) until the end of the trial at 18 DAH. Two microdiets were tested in triplicate tanks: diet Low (commercial-like diet with low molecular weight protein hydrolysates) and diet High (commercial-like diet with high molecular weight protein hydrolysates). For the assessment of performance, larvae were sampled at 2, 12, 16 and 18 DAH for weight and length. Survival was determined at 18 DAH. A 24 h thermal challenge was conducted from 16 to 17 DAH, where the temperature of the water was increased 5ºC compared to standard conditions, to evaluate the larvae resilience. At 16 and 17 DAH, physiological biomarkers of oxidative stress (catalase, superoxide dismutase, total antioxidant status and lipid peroxidation) were assessed. Additionally, the expression levels of genes related to digestion (*pept1*, *ialp*, *tryp*), antioxidant defence (*sod*, *cat*, *gpx4*, *nrf2*), and cellular stress (*hsp60*, *hsp70*) were evaluated.

**Results and Discussion**
Biometric data (weight, length and condition factor) showed significantly improved larval performance up to 12 DAH in the High treatment. At 16 DAH, the Low larvae showed better performance indicators. It is known that the digestive capacity of sole decreases during the climax of metamorphosis (Canada et al., 2017; Engrola et al., 2009). The observed shift in performance relative to peptide size suggests that larvae between 12 and 16 DAH benefited from a diet containing lower molecular weight peptides during this transitional phase.

Oxidativestress biomarkers did not vary significantly across groups or sampling points, regardless of diet or thermal challenge. No significant differences in gene expression levels were observed at 16 DAH. However, sole fed the Low diet showed a significant upregulation of *cat, nrf2, pept1* and *hsp60* following the thermal challenge at 17 DAH. This transcriptional response suggests that low molecular weight hydrolysates may enhance cellular readiness to cope with oxidative and thermal stress by supporting antioxidant defence (*cat*, *nrf2*), nutrient uptake (*pept1*), and protein homeostasis (*hsp60*).

These results highlight the potential of tailored diets to improve growth of Senegalese sole during the metamorphosis, as well as enhancing stress resilience during early developmental stages in marine fish larvae.

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**Acknowledgements**
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# ROLE OF IGF-1 AND IGF-2 IN LOCAL IGF SYSTEM AND ASSOCIATED SIGNALLING PATHWAYS OF CULTURED MUSCLE SATELLITE CELLS IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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## Introduction

In fishes, axial musculature accounts for 60–70 % of body weight and is largely composed of white muscle fibres, which are of high commercial interest. Unlike in mammals, these fibres are generated during myogenesis, a process that can form new muscle fibres even after sexual maturity in several teleosts, such as E. sea bass. This myogenic process is driven by satellite cells, which play a crucial role in continued muscle growth; they proliferate, differentiate, and fuse into myotubes through the action of myogenic regulatory factors (MRFs) such as myod, myf5, myogenin, and mrf4. Growth is also regulated by the GH/IGF axis, in which growth hormone (GH), secreted by the pituitary, stimulates the liver to produce insulin-like growth factors (IGFs), which act in target tissues to stimulate growth via the PI3K–Akt–mTOR and MAPK pathways. The IGF system comprises IGF-1 and IGF-2, their receptors (IGF-1ra, IGF-1rb), and binding proteins (IGFBPs). Therefore, this study aims to determine the effects of IGF-1 and IGF-2 under optimal proliferation conditions, analyse the expression of key markers at the proliferation (day 5) and differentiation (day 8) stages, and evaluate differences in the proliferative effects of IGF-1 and IGF-2 on E. sea bass myogenic cultures.

## Material and methods

E. sea bass fingerlings (10 ± 3 g; Culmarex, Murcia, Spain) were used to establish primary myocyte satellite cell cultures from epaxial muscle. Cells were seeded at $7.5 \times 10^5$ cells/mL in 6 well plates coated with poly-L-lysine followed with laminin and maintained at 23 °C and 2.5 % $CO_2$. Seven independent cultures were used for 5-bromo-2□-deoxy-uridine (BrdU) proliferation assays in 96 well plates, each condition in triplicate. After 12 h of fetal bovine serum (FBS) starvation, cells (day 4) were treated with IGF-1 from chicken (CH1; 100, 500 ng/mL), IGF-1 from sea bream (SB1; 100, 500 ng/mL), or IGF-2 from chicken (CH2; 100, 500 ng/mL), plus controls with and without FBS (10 % or 0 %). BrdU (10 $\mu$M) was added after 5 h, and cells were fixed 5 h later; the immunofluorescence detection was performed according to the manufacturer's protocol (11 296 736 001; Roche, Basel, Switzerland). The most proliferative dose for each IGF was then tested in 6 well plates to assess growth-related gene expression at days 5 and 8 (controls, CH1 and SB1 at 100 ng/mL, CH2 at 500 ng/mL), following 12 h of FBS starvation and 24 h of treatment.

## Results and discussion

BrdU assays revealed an increased proportion of labelled nuclei in treated cells with 100 ng/mL IGF-1 (CH1 and SB1), while the same statistical significance required a concentration of 500 ng/mL in IGF-2 treated cells (Figure 1). This may indicate that lower levels of IGF-1 are sufficient to promote proliferation. Additionally, *igf-1* transcript levels were low at both day 5, and 8, in contrast to *igf-2*, which showed higher expression, suggesting a predominantly proliferative role for IGF-1 and a differentiation-oriented role for IGF-2, in contrast to what has been reported for sea bream Azizi et al., 2016).

During peak proliferation (day 5), cells treated with IGFs showed a slight increase in *pcna* expression, possibly reflecting enhanced cell proliferation activity. In contrast, *myod1* expression was significantly downregulated in all IGF treatments, whereas *myod2* expression was higher, which may indicate that IGF treatment accelerated the myogenic programme and promoted more proliferation. On the same day, *igfbp-3a* and *igfbp-5b* were also upregulated in CH1 and CH2, suggesting a possible regulation mechanism aimed at modulating IGF bioavailability. No significant differences were observed for the

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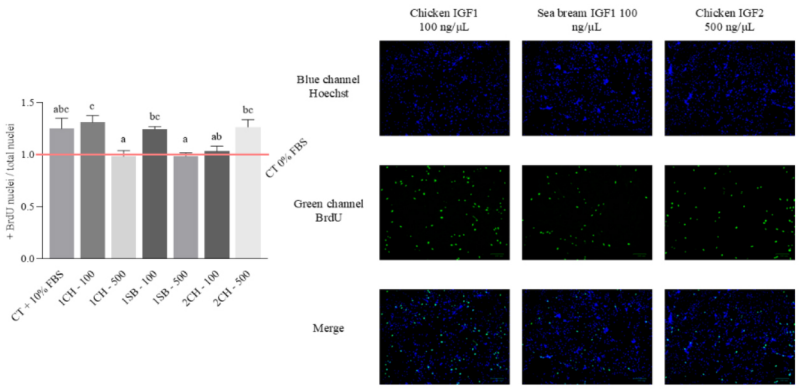


**Figure 1:** BrdU-positive nuclei relative to total nuclei stained with Hoechst in E. sea bass myocyte cultures treated with the selected doses of IGF-1 from chicken (CH1, 100 ng/mL), IGF-1 from sea bream (SB1, 100 ng/mL), and IGF-2 from chicken (CH2, 500 ng/mL). Representative images show Hoechst staining (blue channel), BrdU labelling (green channel), and merged images.

remaining genes at this stage. As cultures progressed to the differentiation phase (day 8), *myod1* expression decreased further compared to day 5, while myod2 showed significant upregulation in all IGF treatments, consistent with increased myoblast differentiation. *Myogenin* expression was slightly higher in IGF-1 and IGF-2 from chicken, while *mrf4* expression and *myhcb* increased overall compared to day 5, likely reflecting progression from proliferation towards late-stage differentiation and maturation of myotubes.

Overall, the results indicate that both IGF-1 and IGF-2 stimulate proliferation and differentiation of E. sea bass white muscle satellite cells, promoting an earlier transition from proliferation to differentiation and involving feedback regulation of IGF bioavailability. At this stage, eif4ebp1 expression in the IGF-1 (chicken) treatment tended to be higher, possibly indicating a role for IGF-1 in promoting differentiation. Core signalling and fusion-related genes remained unaffected, and both IGFs displayed proliferative effects, although IGF-2 required a higher concentration to match IGF-1 responses, supporting their potential use to enhance muscle growth efficiency in E. sea bass myocytes primary culture.

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# EFFECTS OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAL MEAL DIET ON REPRODUCTIVE PERFORMANCE AND HEALTH INDICATORS IN AFRICAN CATFISH (*Clarias gariepinus*) BROODSTOCK

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**Introduction**

Successful aquaculture relies on healthy broodstock, which ensures high-quality gametes. Nutrition plays a critical role in reproductive performance and has recently shifted toward more sustainable protein sources (Migaud et al., 2013). Fishmeal is increasingly being replaced by alternatives such as insect meals. While insect meal has been widely studied for growth and meat quality (Alfiko et al., 2022), little is known about its effects on broodstock reproductive traits and gamete quality. Limited studies in species like zebrafish and brown trout have shown mixed results (Chemello et al., 2022). Thus, the long-term reproductive effects of insect-based feeds remain largely unknown.

**Materials and methods**

Freshly hatched African catfish (*Clarias gariepinus*) larvae were fed Artemia nauplii for the first five days post-hatch. Following this initial period, experimental diets were introduced. The Control group received only commercial feeds, while in the BSF33 group, 33% of the feed was replaced with Black Soldier Fly (*Hermetia illucens*) (BSF) meal, and in the BSF50 group, 50% of the feed was substituted with BSF meal. Fish were maintained in a continuous recirculating aquaculture system (RAS) at a constant temperature of 25 ± 1 °C.

To ensure optimal broodstock selection, continuous growth measurements and phenotypic selection were carried out throughout the rearing period. After 18 months of culture, 5 males and 5 females from each group were selected and used for propagation. Individual body weight and stripped egg quantity were recorded. Additionally, the weight of the testes and liver was measured for each individual.

The number of eggs per gram was also determined by manually counting the eggs in a 1-gram subsample of stripped eggs. Based on these measurements, the gonadosomatic index (GSI) was calculated as gonad weight / body weight × 100, and the hepatosomatic index (HSI) as liver weight / body weight × 100.

**Results**

No significant differences were observed among the dietary groups in terms of body weight, egg size, gonadosomatic index (GSI) in either sex, or hepatosomatic index (HSI). All measured parameters remained statistically similar across the Control, BSF33, and BSF50 groups (Table 1.).

**Discussion**

In our experiment, 33% and 50% of the commercial feed was replaced with black soldier fly larvae (BSFL) meal. We examined the effects of the different diets on egg diameter, the gonadosomatic index (GSI), and the hepatosomatic index (HSI). Even at the highest level of BSFL inclusion, no statistically significant differences were observed. The values obtained for egg size (Hogendoorn, 1980; Opiyo et al, 2017), GSI (Mikaheel et al., 2019), and HSI (Ajang et al., 2018) were consistent with those reported in the literature.

**Conclusion**

Replacing 33% and 50% of the complete feed with black soldier fly larvae meal has no negative effect on egg size, GSI, or HSI.

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1. **Table:** Reproductive and physiological parameters of African catfish broodstock fed diets with varying levels of Black Soldier Fly (BSF) meal inclusion.

| | C | BSF33 | BSF50 |
|---|---|---|---|
| Body weight (g) | 2978,60±610,89[a] | 3122,70±776,72 [a] | 2973,70±255,90 [a] |
| Egg size (egg/g) | 842,75±33,57 [a] | 844,60±38,29 [a] | 857,25±43,12 [a] |
| GSI female (%) | 9,84±2,55 [a] | 9,93±3,77 [a] | 11,85±2,36 [a] |
| GSI male (%) | 0,76±0,78 [a] | 0,85±0,39 [a] | 0,55±0,40 [a] |
| HSI (%) | 1,18±0,18 [a] | 1,00±0,15 [a] | 1,03±0,13 [a] |

**Acknowledgement**

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# OPTIMIZATION OF A MULTICOLOR TRANSGENIC BIOMARKER ZEBRAFISH LINE ASSAY FOR THE *IN VIVO* DETECTION OF XENOESTROGENIC COMPOUNDS

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**Introduction**

Endocrine-disrupting chemicals (EDCs), particularly estrogenic xenoestrogens, are prevalent environmental pollutants that accumulate in surface waters, posing serious ecological and health risks. These compounds can cause feminization, altered sex ratios, reduced fertility in wildlife, and reproductive, immunological, cardiovascular, and oncogenic effects in humans. Due to their chemical diversity, EDCs require varied analytical approaches, and their estrogenic activity cannot always be predicted from structure alone. In nature, they usually occur as complex mixtures whose biological effects are influenced by concentration, solubility, degradation rate, microbial transformation, and environmental conditions. These factors complicate purely chemical detection methods, making bioassays with biomarker organisms a valuable alternative.

Fish are especially suitable vertebrate models for environmental risk assessment. They absorb contaminants across their entire body surface, have short generation times, high fecundity, and share key endocrine pathways with other vertebrates, including humans. Several estrogen-responsive genes have been characterized in fish and used to develop transgenic *in vivo* biosensors, such as zebrafish *(Danio rerio)* reporter lines. Since xenoestrogens affect multiple biological pathways, reliable assessment often requires combined *in vitro* and *in vivo* approaches, which can be time- and resource-intensive and may conflict with the 3R principle. Multicolor transgenic reporter systems, such as multicolor zebrafish lines, offer a promising alternative but remain scarce.

This study aimed to develop a novel multicolor transgenic zebrafish line (Tg(vtg1:mCherry) × Tg(cyp19a1b:GFP)) enabling simultaneous *in vivo* monitoring of vitellogenin- and aromatase-pathway activation. We adapted the EASZY protocol for aromatase analysis to the characteristics of this new line and designed experimental procedures to measure transgene expression in parallel. We tested the estrogenic activity of 17β-estradiol (E2), the fungicide prochloraz, and an water/aqueous extract of Hytrel plastic using transgenic embryos.

**Materials and Methods**

The new biomarker line was created by crossing Tg(cyp19a1b:GFP) and Tg(vtg1:mCherry) zebrafish, selecting F1 individuals carrying both transgenes after 50 µg/L E2 induction. Positive embryos were raised, in-crossed to produce the F2 generation, and characterized by documenting fluorescence development from 1–5 days post-fertilization (dpf) under brightfield, GFP, and mCherry filters. Images were analyzed using ImageJ to calculate integrated density (ID) and GFP fold-change values.

For short-term exposure assays, embryos at the 8-cell stage were treated with E2 (1–200 µg/L), prochloraz (17.5–300 µg/L), or Hytrel extract (4.8 mg/L) until 5 dpf. Imaging conditions (orientation, magnification, and timing) were optimized for each reporter: lateral views for liver-localized mCherry (vtg1:mCherry expression) and dorsal views for brain-localized GFP (cyp19a1b:GFP expression).

*(Continued on next page)*

**Results and Conclusions**

The developmental atlas showed mCherry expression in the liver from day 4, peaking at day 5, and GFP expression in the brain from day 3. Optimal simultaneous detection of both reporters was achieved at 5 dpf, slightly modifying the EASZY protocol's 4-day exposure. Reporter signals were orientation-dependent: lateral imaging best captured liver fluorescence, while dorsal imaging was superior for brain signals.

Prochloraz reduced cyp19a1b expression, indicating anti-estrogenic activity, while Hytrel also suppressed aromatase expression, possibly due to toxicity. Both reporters displayed similar estrogen-sensitivity patterns. The differences in imaging requirements for examining embryos containing the two transgenes support the continued use of dual-orientation imaging in future studies.

**Summary**

We established a multicolor transgenic zebrafish line that combines vitellogenin- and aromatase-based estrogen reporters, enabling rapid and reliable detection of estrogenic or anti-estrogenic activity in a single assay. This system reduces the number of experimental animals required, increases efficiency, and can be applied to diverse chemicals, mixtures, and environmental samples, contributing to improved environmental risk assessment and endocrine toxicology.

**Acknowledgement**

The work was supported by the 'PFAQuatic' 2024-1.2.3-HURIZONT-2024-00100 and the 2024-1.2.1-HE_PARTNERSÉG-2024-00024 "BioTreatED", the professional assistance of the University Research Fellowship Program (code: EKÖP-MATE/2024/25/D) of the Ministry of Culture and Innovation, funded by the National Research, Development and Innovation Fund projects, as well as by the MATE Research Excellence Program (ZCB).

# SHELF LIFE MONITORING OF GILTHEAD SEABREAM FILLETS *Sparus aurata* USING BIOGENIC SMART PACKAGING MATERIALS

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## Introduction

Significant amounts of fish and seafood are wasted worldwide due to spoilage and degradation at various stages of the cold chain (EUMOFA, 2022). The development of innovative smart packaging solutions for monitoring the deterioration of fish products could provide consumers with reliable indicators of product freshness, thereby contributing to reduction in food waste (Tsironi et al., 2018; Li et al., 2022). This study focused on the design of freshness indicators responsible for volatile ammonia compounds, facilitating the monitoring of spoilage within fish packaging environments through visible color changes. The smart packaging systems were formulated using starch and polyvinyl alcohol, enriched with anthocyanins derived from pomegranate juice. The objective was to develop biodegradable, environmentally friendly packaging that delivers real-time visual information on the quality and deterioration of packaged fresh fish.

## Materials and methods

The smart packaging system was prepared according to the solvent casting method, incorporating starch and polyvinyl alcohol (PVA) with pomegranate juice added as a natural source of anthocyanidins (Alizadeh-Sani et al., 2020). Aiming at a sustainable production process for the smart indicators, the anthocyanidins from the pomegranate juice were not isolated to eliminate the need for organic solvents and energy-intensive processes. Following preparation, the smart films were characterised for their optical properties, microstructure (SEM), infrared spectrum (FTIR), wettability, water solubility, moisture adsorption (sorption isotherms), color response at various pH values and reactivity to volatile amines commonly produced during fish spoilage, such as ammonia, trimethylamine and dimethylamine. The smart indicators were subsequently placed inside packages containing gilthead seabream (*Sparus aurata*) fillets. The samples were stored under controlled temperature conditions (4°C) for shelf life evaluation based on chemical (pH, TVB-N) and microbiological fish quality indicators (total viable counts, *Pseudomonas* spp. and Enterobacteriaceae). The color change of the indicators (ΔE value) and the gas composition in the package headspace were continuously monitored during the storage experiment.

## Results and Discussion

Scanning electron microscopy revealed satisfactory microstructural integrity and compatibility between the incorporated dye and the biopolymer carrier. Successful incorporation of the dye was further confirmed through Fourier transform infrared spectroscopy. The developed films exhibited suitable optical and mechanical properties for potential packaging applications. The incorporation of pomegranate juice enhanced the hydrophilicity of the membranes (contact angle reduction from 93° to 82°). The color sensitivity test demonstrated that the color response of smart films successfully monitored the increase in volatile amine concentration and pH values, exhibiting a visible color shift from bright red to purple-green. Preliminary results of the application of the freshness indicators within the headspace of packaged gilthead seabream fillets demonstrated their capability to monitor the accumulation of volatile amines and the progression of fish spoilage during refrigerated storage. A perceptible color change (ΔE>3) was observed from the early stages of spoilage (day 4-5), with intensity increasing as degradation advanced. The indicators showed a reliable correlation with changes in headspace gas composition and were consistent with microbiological and chemical quality indicators of fish flesh, highlighting their potential as effective tools for real-time spoilage monitoring.

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# BLACK SOLDIER FLY LARVAE OIL AS A FUNCTIONAL INGREDIENT FOR EUROPEAN SEABASS JUVENILES: RESPONSE TO AN ACUTE STRESS CHALLENGE

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**Introduction**

Black soldier fly (BSFLO), a by-product of insect meal processing, is characterized by presenting high levels of lauric acid (30-40%), a fatty acid suggested to enhance hepato-metabolic status. Additionally, when sourced from larvae-fed vegetable wastes rich in tocopherols, BSFLO also presents significant levels of these antioxidant compounds. Stress in aquaculture fish, often triggered by factors such as overcrowding, poor water quality, or sudden environmental changes, can significantly impair their immune function, growth rates, and overall welfare. This underscores the critical need for feeding strategies that not only meet the nutritional requirements but also help mitigate stress. Therefore, this study aims to appraise the impact of BSFLO dietary inclusion on the metabolic and oxidative response of European seabass (*Dicentrarchus labrax*) juveniles – a key species in Mediterranean aquaculture – following an acute stress challenge.

**Material and methods**

A control diet (CTRL) was formulated with 6% fish oil and 5% poultry oil (PO). Three other experimental diets were formulated to include 1.7%, 3.4% and 5.2% of BSFLO, supplied by Entrogreen® - Ingredient Odissey S.A. (BSFLO1.7, BSFLO3.4 and BSFLO5.2, respectively), at the expense of PO. Each diet was assigned to triplicate homogeneous groups of 50 fish (11 ± 0.1 g) that were fed until apparent satiation three times daily for 12 weeks. Fish were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating saltwater system (35‰, 23 ± 1 °C). By the end of the trial, 5 fish per tank (15 per dietary treatment) were sampled for analysis of metabolic and oxidative stress response-related parameters, and other 5 fish per tank were exposed to an acute stress episode consisting of 1 min air exposure and 14 min overcrowding (100 kg/m$^3$) followed by 90 min of recovery before sampling. Plasma cortisol levels, along with plasmatic and hepatic metabolites (e.g., glucose, lactate, triglycerides, among others) were quantified. Additionally, lipid peroxidation and the activity of oxidative stress-related enzymes (e.g., superoxide dismutase, catalase, and glutathione peroxidase, and others) were measured. Growth performance and feed efficiency were also evaluated.

**Results**

The experimental diets ensured a 100% survival rate, and no behavioural alterations were observed in the fish throughout the 12-week feeding trial. Fish fed the BSFLO5.2 diet achieved the highest final body weight and the lowest feed conversion ratio (FCR). A clear response to acute stress was observed, as indicated by significantly increased levels of plasmatic cortisol, glucose and lactate, as well as lipid peroxidation products in the liver of fish sampled after the acute stress challenge. Based on the Mahalanobis distance between group centroids, fish fed BSFLO5.2 diet were clustered at a significant distance from the other groups either before or after stress challenge (Fisher's $p$-value < 0.0001), with this separation being particularly prominent between the BSFLO5.2 and CTRL groups (Figure 1). Indeed, based on the two-way ANOVA, fish fed BSFLO5.2, specifically after the stress challenge, exhibited significantly lower (Tukey's $p$-value < 0.05) plasma cortisol (Figure 2-A) and cholesterol levels than those fed CTRL. Additionally, BSFLO5.2 group also had significantly lower lipid peroxidation (LPO) (Figure 2-B) and superoxide dismutase (SOD) activity compared to CTRL. Glutathione peroxidase (GPx) activity was also the lowest in seabass fed BSFLO5.2, regardless of stress condition. Although without statistically significance, stressed fish fed BSFLO5.2 also have lower levels of plasma glucose, lactate, and triglycerides, as well as hepatic cholesterol, non-esterified fatty acids (NEFA), and catalase (CAT) activity compared to CTRL-fed fish.

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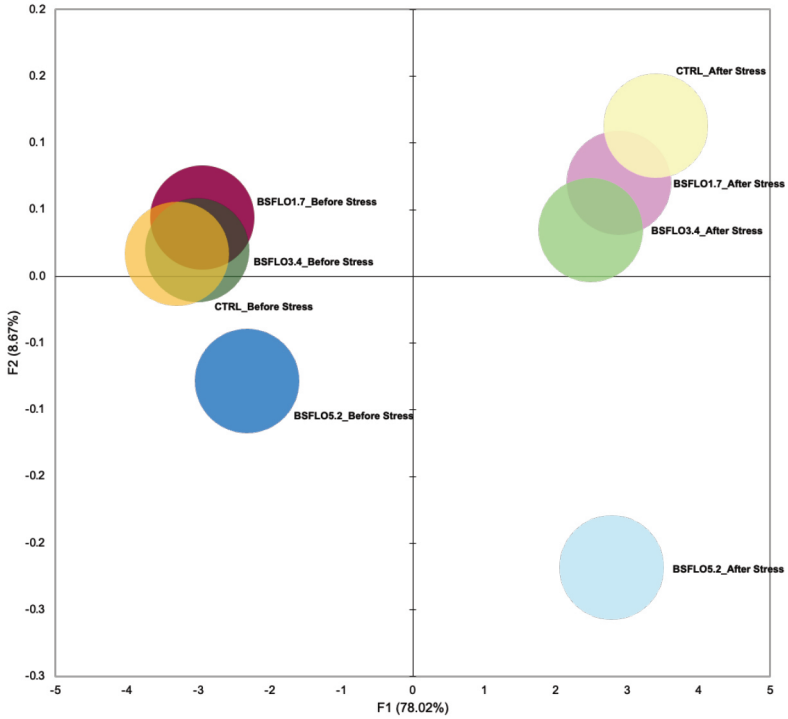


**Figure 1.** Canonical discriminant analysis of European seabass fed experimental diets, before and after stress.

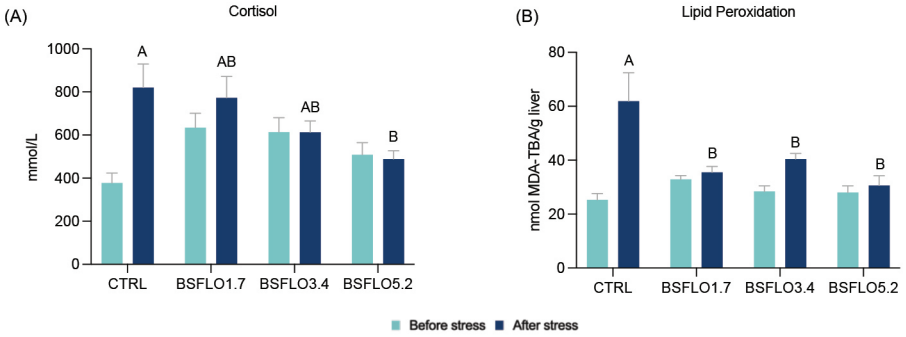


**Figure 2.** Plasma cortisol levels (A) and hepatic lipid peroxidation (B) in European seabass fed experimental diets, before and after stress.

## Discussion and conclusions

European seabass juveniles fed the BSFLO5.2 diet exhibited a marked reduction in cortisol levels - the primary indicator of stress - as well as decreased plasma cholesterol, its metabolic precursor. This attenuation of the stress response can be associated with reduced activation of key antioxidant enzymes, including SOD and GPx, and lower levels of LPO, suggesting diminished oxidative stress. In addition to these physiological benefits, the 5.2% inclusion of BSFLO significantly enhanced growth performance and feed efficiency. Together, these findings indicate that BSFLO, particularly when included at 5.2%, can modulate stress-related pathways while supporting optimal growth in European seabass juveniles, highlighting its potential as a functional ingredient in aquafeeds.

## Acknowledgments

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# PILOT-SCALE CULTIVATION AND GROWTH ASSESSMENT OF A RANDOM MUTAGENESIS-DERIVED *Phaeodactylum tricornutum* STRAIN FOR AQUACULTURE APPLICATIONS

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## Introduction

In recent decades, microalgae have gained recognition as high-value biological resources due to their ability to produce a wide array of bioactive compounds with diverse applications in aquaculture, pharmaceuticals, cosmetics, agriculture, food, and feed[1]. This biotechnological potential has driven continuous efforts to enhance microalgal biomass productivity and metabolite accumulation through both cultivation optimization (e.g., light, temperature, nutrients, CO□) and genetic approaches such as metabolic engineering and selective breeding[2-3]. In parallel, random mutagenesis has emerged as a well-established, low-cost and relatively straightforward strategy to generate novel microalgal strains with improved phenotypes, without requiring prior genomic knowledge[4]. Moreover, it enables the selection of mutants even when underlying metabolic pathways are poorly understood. As non-transgenic variants, random mutagenesis-derived mutants face no regulatory restrictions for large-scale application in opposition to genetically modified organisms[4]. These advantages make it a valuable tool to create strains with desirable attributes, such as accelerated growth or high biometabolite output, suitable for industrial cultivation. Among microalgae, the marine diatom *Phaeodactylum tricornutum* stands out for its fast growth, ease of cultivation, and ability to synthesize high-value compounds such as fucoxanthin and omega-3 fatty acids[5]. It is widely used as a nutritious feed in aquaculture, providing a high protein content (≈ 49% of biomass) and accumulating up to ≈ 35% EPA, which is essential for fish larvae and molluscs[6]. Beyond aquaculture, *P. tricornutum* is also considered a promising production platform for market-relevant bioproducts in the pharmaceutical and nutraceutical sectors[5]. Nevertheless, maximizing *P. tricornutum* productivity under large-scale cultivation remains a challenge, underscoring the importance of strain improvement and process optimization to enhance economic feasibility and competitiveness. In this context, the present study aimed to assess the growth performance and biomass productivity of a *P. tricornutum* mutant strain (MT009), obtained through random mutagenesis, in comparison with the wild-type (WT) strain when cultivated in pilot scale flat-panel photobioreactors.

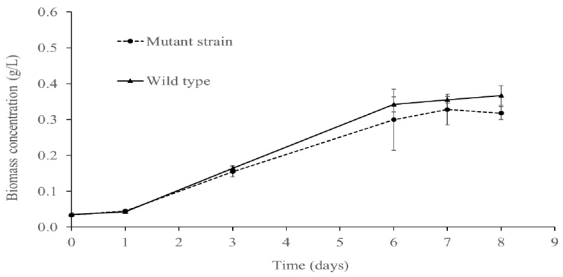


**Figure 1**: Biomass concentration (g $L^{-1}$ DW) of MT009 and wild-type *P. tricornutum* strains over 8 days of outdoor cultivation in 100 L flat-panel photobioreactors (mean ± SD, $n = 3$).

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**Material and Methods**

The *P. tricornutum* mutant strain (MT009) was generated through chemical mutagenesis using ethyl methanesulfonate (EMS) at a final concentration of 200 mM for 60 minutes under constant agitation. In a previous screening assay, MT009 and WT strains were cultivated under different salinities (5, 20 and 35 ppt) and temperature conditions to select the best cultivation conditions. Cultivation at 33 ppt of salinity and 28ºC was selected. The MT009 and WT *P. tricornutum* strains were subsequently scaled up from 250 mL to 1 and 5 L round-bottom flasks under controlled temperature, and then cultivated for 8 days in 100 L outdoor flat-panel photobioreactors (FP-PBRs) operated in fed-batch mode. Finally, each culture was split into three 100 L FP-PBRs ($n$ = 3). All FP-PBRs cultures were exposed to natural light and ambient temperature (average temperature of approximately 18ºC), with continuous mixing provided by bubbling aeration. The pH was maintained around 8.2 through periodic $CO_2$ injection. The culture medium consisted of UV-sterilized, ultrafiltered natural seawater enriched with NutriBloom® Plus. Growth and nitrate uptake were monitored daily by measuring optical density (OD) at 540 and 220/275 nm, respectively. Biomass concentration (dry weight, DW), was estimated using $OD_{540}$-to-DW calibration curves. Biomass productivity (g $L^{-1}$ $day^{-1}$) was calculated from DW and cultivation time, and the specific growth rate (μ) was determined during the exponential phase using: $\mu = [\ln(N_2) - \ln(N_1)]/[t_2 - t_1]$, where $N_1$ and $N_2$ are biomass concentrations at times $t_1$ and $t_2$, respectively.

**Results and Discussion**

Both strains were scale-up in 100 L FP-PBRs to ensure equivalent inoculation conditions, sufficient biomass, and physiological acclimation to the cultivation system. At the end of this scale-up, the MT009 and WT strains had reached 0.28 and 0.21 $gL^{-1}$ DW, respectively. The cultures were then split into three 100 L FP-PBRs to initiate a 8-day comparative trial. Both cultures displayed steady exponential growth from day 1 to 6 (Fig. 1), culminating in final biomass concentrations of 0.32 ± 0.02 $gL^{-1}$ for the mutant and 0.37 ± 0.03 $gL^{-1}$ for the WT. The WT exhibited a slightly higher specific growth rate (μ = 0.414 $day^{-1}$) than the mutant (μ = 0.377 $day^{-1}$), suggesting a marginal advantage in growth kinetics under the tested conditions. Although modest, this difference may reflect subtle variations in cellular metabolism, light-use efficiency, or nutrient assimilation. Nonetheless, the mutant followed a similar growth trajectory, reaching a peak biomass productivity of 0.0546 g $L^{-1}$ $day^{-1}$ on day 3 compared to 0.0606 g $L^{-1}$ $day^{-1}$ for the WT. WT reached stationary phase on day 6, and MT009 strain on day 7 as indicated by biomass stabilization and productivity decline, likely due to nutrient depletion, self-shading, or metabolite accumulation.

In conclusion, the *P. tricornutum* MT009 strain exhibited growth and biomass productivity comparable to the WT under pilot-scale conditions, reinforcing random mutagenesis as a viable and scalable tool for microalgal strain improvement. Ongoing characterization of its biochemical composition and additional pilot-scale trials at higher temperatures will further validate MT009 strain thermotolerance and clarify its robustness and potential for large-scale commercial deployment.

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# DEPURASHELL: A NOVEL MICROBIOLOGICALLY-SAFE MICROALGAE DIET FOR ENHANCED RELAYING AND DEPURATION OF THE OYSTER *Magallana gigas*

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## Introduction

Bivalves harvested or reared in areas with high microbiological contamination require depuration in clean seawater without food for 1–3 days, or longer in relaying facilities, to ensure safety for human consumption[(1)]. However, this starvation period can impair survival, physiological condition, health, and nutritional value, challenging their marketability[(1)]. To mitigate these effects, safe and nutritionally balanced microalgae-based diets for depuration are essential for enhancing bivalve welfare, quality, and producers' revenues. However, the production of live microalgae in hatcheries for bivalve nutrition represents 30-50% of total operational costs[(2)] and poses logistical and technical challenges, driving the pursue for alternative and cost-effective commercial microalgal feed solutions. Microalgae liquid concentrates have emerged as a promising option, offering several advantages such as ready-to-use diets, year-round availability, consistent quality, and nutritionally adequate profiles. However, existing commercial products hinder their application in relaying and depuration since they are not adapted to these aquaculture systems and contain high microbial loads, which can compromise their technical application, as well as feed safety, stability, shelf-life, and bivalve performance. High microbiological loads in diets degrade water quality, disrupt organisms' gut microbiota, and weaken their immune system[(3)]. Therefore, improving the microbial quality of these diets is crucial, especially for depuration, which requires bacteriologically clean water[(1)]. Thermal treatments efficiently reduce the bacterial load in microalgae liquid concentrates, making them promising for use in depuration system diets[(4)]. This study aims to develop a novel microbiologically-safe, nutritionally balanced microalgae diet (DepuraShell) to improve the quality of Pacific oysters (*Magallana gigas*) during depuration, while being technically adapted to Recirculation Aquaculture Systems (RAS). For that purpose, the organism's biological performance was evaluated during depuration and shelf-life storage, focusing on oysters' physiological status.

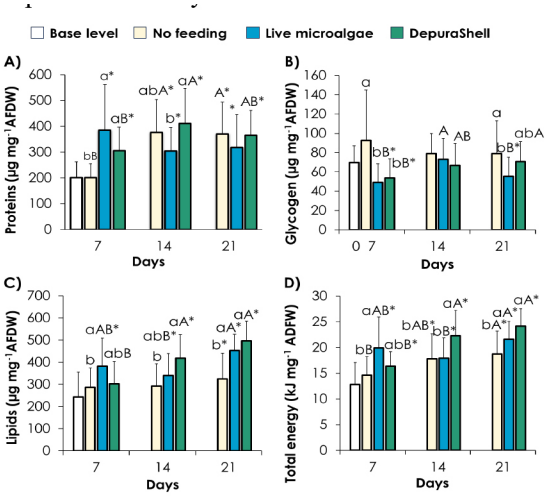


**Figure 1**: *Magallana gigas* oysters proximate composition after 21 days of depuration by dietary treatment (mean ± SD, $n = 20$): A) protein, B) glycogen, C) lipids and D) total energy. Asterisks indicate significant differences from base level (independent samples *t*-test, $p < 0.05$). Lowercase letters show significant differences between dietary treatments at each timepoint, while uppercase letters show significant differences over time within the same treatment (one-way ANOVA, *post-hoc* Tuckey, $p < 0.05$)

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**Material and Methods**

The previously selected strategy to lower the bacterial load of microalgae liquid concentrates was low-temperature long-time (LTLT) pasteurization (75±2ºC, 1h, followed by a 10ºC cold shock) due to its efficiency in lowering bacteria, without causing cell agglomeration, sedimentation, or degradation of key pigments. The LTLT-treated DepuraShell pilot diet was formulated as a liquid concentrate with 8% algal dry weight (DW), containing *Tetraselmis chui*, fortified with docosahexaenoic acid (DHA), and supplemented with carbohydrate-rich ingredients. LTLT was applied to the diet and frozen until its usage. A 28-day trial was conducted in three closed 250L depuration modules with ultrafiltered and UV-treated recirculating seawater (15°C, salinity 35), each equipped with a protein skimmer. Oysters (*n*=354) were assigned to three feeding treatments: (1) live microalgae (*Tisochrysis lutea* + *Tetraselmis* sp., 3:2 ratio; (3% of oyster DW (g) in algal DW (g)/day), (2) LTLT-treated DepuraShell diet; (8% of oyster DW (g) in diet DW (g)/day), and (3) an unfed control. Oysters were fed twice daily for 21 days, followed by 7 days of storage at 4°C (shelf-life simulation). Daily survival was recorded, *Escherichia coli* levels (Most Probable Number, MPN) were quantified at 0 and 18h, and weekly assessments included condition index (CI), proximate composition (proteins, glycogen, lipids, total energy), and biometric parameters (wet weight, length, width, thickness). Fatty acids profiles were analysed at day 0, day 21, and day 28.

**Results and Discussion**

Results showed effective depuration in all treatments, with *E. coli* levels dropping from 1300 to <18 MPN/100g within 18h. Survival remained near 100% across all treatments throughout the 21-day trial. Oysters fed with DepuraShell diet exhibited significantly higher shell length (91.26±9.46 mm) and wet weight (74.38±7.86 g) compared to the base level. During this period, DepuraShell also significantly improved oyster's CI compared to the unfed control, with no significant differences compared to live microalgae. DepuraShell-fed oysters accumulated significantly higher lipid (496±89.66 $\mu g.mg^{-1}$ ash free dry weight (AFDW)) and total energy reserves (24.14±3.45 $kj.mg^{-1}$ AFDW) at day 21, compared to unfed oysters, mitigating the typical lipid depletion observed in food-deprived bivalves during the depuration process. Glycogen reserves were mobilized in fed groups due to higher metabolic activity, in contrast to the unfed condition, where lower metabolic activity occurred due to the absence of feed. These oysters also exhibited significantly higher DHA and eicosapentaenoic acid (EPA) levels (26.94±0.66; 15.45±0.75% of total fatty acids, respectively), and had significantly higher *n*-3/*n*-6 (9.53±0.72), DHA/EPA (1.75±0.12), and PUFA/SFA (1.85±0.08) ratios compared to the unfed group, making it an excellent alternative to standard depuration without feeding. During the 7-day shelf-life period, survival remained at 100%, and oysters' CI, along with protein, lipid, and total energy levels, were maintained in DepuraShell-fed oysters.

In conclusion, DepuraShell is a nutritionally balanced diet, technically adequate for RAS systems, that enhances *M. gigas* commercial value during relaying and depuration by improving growth, body condition, nutritional quality and fatty acid profile, while guaranteeing microbiological safety for human consumption.

**Acknowledgements**

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# INTEGRATING *Ostrea edulis* TO IMTA SYSTEMS IN ITALY: INSIGHTS FROM GROWTH, PROXIMATE COMPOSITION, AMINO AND FATTY ACID PROFILES

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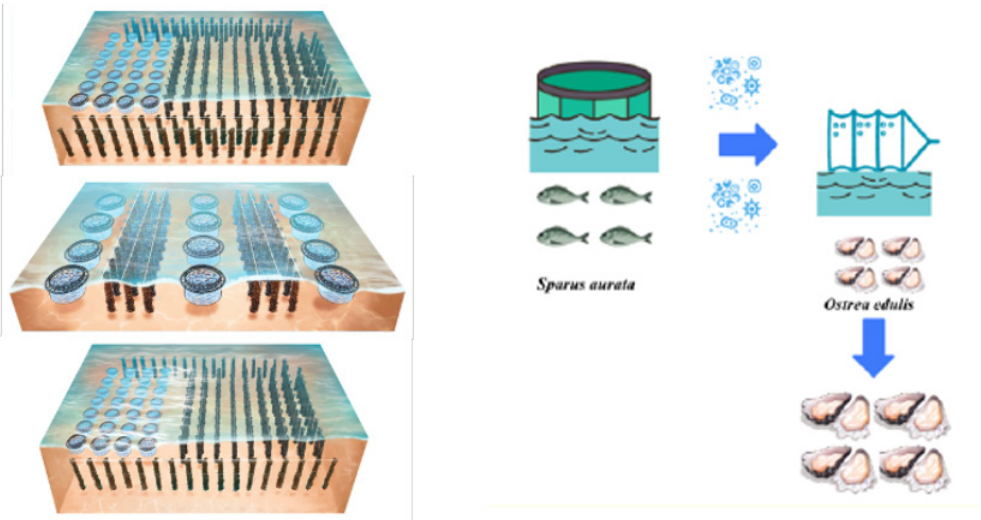
The European flat oyster (*Ostrea edulis*), a filter-feeding bivalve and a high-value seafood product, faces significant challenges in cultivation, including limited production and ecological pressures. To explore its potential in ocean restoration and Integrated Multi-Trophic Aquaculture (IMTA) systems, a 390-day experiment was conducted (February 2023–March 2024) in Gaeta, Italy, in the Tyrrhenian Sea. Oysters were deployed in lantern nets at two locations: 20 m from fish farm cages (IMTA site) and 600 m away (Mono site). Sampling occurred bi-monthly, assessing growth parameters, specific growth rates (SGR), nutritional composition, amino and fatty acid profiles.

Initial mean weights of oysters (28.31±2.00 g), shell length (7.04±0.70 cm) and shell area (23.84±1.36 $cm^2$) increased to 49.41±2.18 g, 8.36±0.05 cm, 34.23±2.69 $cm^2$ in the IMTA site and 52.65±2.76 g, 8.32±0.90 cm, 34.94±1.42 $cm^2$ in the Mono site, respectively. Significant seasonal fluctuations in dry meat weights were observed, with oysters at the Mono site showing notably higher values by the end of the experiment. The specific growth rate (SGR) for shell length was 0.10% and for dry weight was 0.22% in both sites.

Nutritional analyses revealed distinct trends, with the highest protein content measured at 7.81±0.21% and lipid levels reaching a maximum of 1.31±0.05%. The Mono site oysters exhibited higher levels of n-3 polyunsaturated fatty acids (PUFAs) and essential amino acids. Seasonal variations were evident, with dry meat and nutrient levels peaking before spawning in April, declining after spawning in November, and increasing again by March in preparation for the next reproductive cycle.

In conclusion, optimal oyster growth was linked to 600 m distances from fish farm, where phytoplankton availability, enhanced by fish farm-derived nutrients, reached optimal levels. Oysters closer to the fish farm struggled with undiluted organic waste, impacting growth efficiency. Based on our findings and existing literature, we developed three productive models considering local hydrodynamics and nutrient distribution for oyster-IMTA. These models offer valuable guidance for Allocated Zone for Aquaculture (AZA) and Marine Spatial Planning (MSP) policies.



# OPTIMISING THE MEASUREMENT WORKFLOW OF GROWTH PARAMETERS FOR AN UPSCALING MACROALGAE INDUSTRY, INTRODUCING PHIAMPP: A POST-HARVEST HIGH-THROUGHPUT IMAGEJ-BASED ANALYSIS WORKFLOW FOR MACROALGAE PHENOTYPING, UTILISING A PHOTOBOX

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**Introduction:**
Over the past thirty years, the macroalgae industry has developed into the fastest-growing aquaculture production, with an annual biomass output of over 35 million tons of fresh weight. However, similar to agriculture, both quantity and quality yields vary significantly between harvests due to various abiotic and biotic factors (Sugumaran et al., 2022, …). Monitoring the status of the crops and distinguishing between potential causes requires highly frequent and precise monitoring of individual macroalgae blade performances. However, while production and cultivation techniques have evolved into large-scale production, growth assessment methodologies have not advanced at the same rate. Furthermore, analysing a statistically meaningful sample size results in substantial working hours, which is amplified if multiple systems and different strains demand monitoring over multiple time points. Moreover, for other potentially vital parameters, e.g., colour, no standardised methodology exists. This creates an informational bottleneck. The automatic measurement of growth parameters, known as plant phenotyping, is a wellestablished field in botany and agriculture. Its importance for the scaling of macroalgae production lines in microscopic and macroscopic stages has been well identified (Overrein et al., 2024, Dishon et al., 2024). First attempts to transfer established imaging technologies to aquatic plants have been conducted for area estimations, percentage calculations, the status of cultures (Overrein et al., 2024) or heat-induced stress on blades (Gonzales et al., 2025, …). While these approaches utilise parts of the image analysis toolkit, there is, to our knowledge, no comprehensive, user-friendly and efficient workflow published for the commonly used ImageJ/Fiji platform, which automatically measures even fundamental growth parameters of individual macroalgae blades and therefore strains. Here, we describe a methodology for both hardware and software, which establishes a semi-automatic analysis for post-harvest samples, utilising in-house developed ImageJ/Fiji, Excel/VBA scripts and standardised light settings. Altogether, these compounds combine existing functionalities in a novel manner to accurately measure relevant growth parameters such as colour, blade area, maximum width and length, per macroalgae blade, for hundreds of macroalgae in one image, while cutting working hours in half.

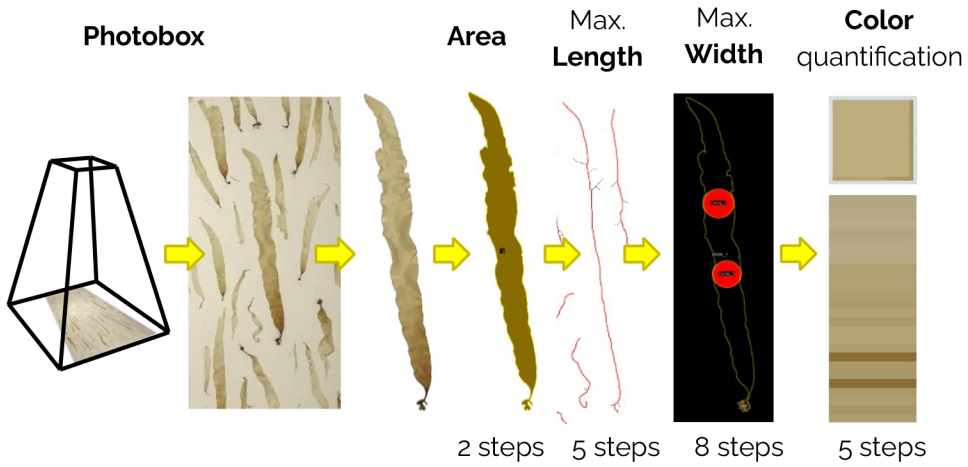


*Figure 1: Workflow of the semi-automatic measuring system PHIAMPP. The setup enables blade-specific measurements of area, width, length and colour. The frequency distribution (top 25 tones) of the blade colours can be visualised as an average, barcode as well as numerically, in HEX and RGB values.*

*(Continued on next page)*

**Methodology:**
**Manual** workflow: … After the harvest of at least 100 individual blades of macroalgae, per length of cultivation substrate, the algae are cooled if not processed immediately. For the picture taking, the algae blades are placed manually onto a white background underneath the custom-built photo box. Length and/or colour scales, as well as metadata, are included.

**Digital workflow:** … custom-written scripts for ImageJ/Fiji and Excel/VBA have been in-house developed to automate almost all necessary steps to measure and visualise the parameters of interest (blade area, maximum length, maximum width and RGB colour), with minimal observer interaction. …

**Discussion:**
While subtle variations in area, length and width were observed, no significant differences between the distribution of the manual and digital datasets were found, indicating a similar precision between the two approaches. This approach enables unprecedented measurements of currently unutilized parameters, such as the quantification and processing of blade colour under standardised light regimes. The workflow has been applied to parts of the *Saccharina ssp*. harvest of the aquaculture research project ULTFARMS, which results in the establishment of a common image database between geographically distant production sites. This increase in standardised image-based data sets will enable further development of advanced algorithms to expand the automated measurement of other relevant, more complex parameters, such as rhizoid width, sorus quantification. Within the near future, we envision a technological advancement in marine plant phenotyping, with similar success as it has been shown for land plants. Further methodological progress will undoubtedly benefit various fields of research in phycology, as it allows for insight into growth patterns relevant for plant health monitoring, pest management and strain performance.

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# OPTIMIZATION OF PHLOROTANNIN EXTRACTION FROM *Cystoseira compressa* USING DIFFEERENT EXTRACTION METHODS

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## Introduction

Phlorotannins are a unique group of polyphenolic compounds, biosynthesized by brown macroalgae (Phaeophyceae) as part of their ecological defense strategies. Built from phloroglucinol monomeric units, they exhibit remarkable structural diversity through different types of linkages and degrees of polymerization, resulting in a wide range of molecular weights and biological activities (Fernando et al. 2022). These activities include potent antioxidant, anti-inflammatory, antimicrobial, antiviral, algicidal effects (Lemesheva et al. 2023), which have attracted considerable attention due to possible uses in the cosmetics, pharmaceutical and nutraceutical sectors. Despite this potential, reliable quantification and bioactivity assessment of phlorotannins remain challenging due to the absence of standardized extraction/fractionation methodologies. Variation in solvents selection, pH adjustment, antioxidant use, and purification techniques contribute to inconsistent yields and hinder significant comparisons between studies.

## Materials and methods

This study aimed to systematically compare and optimize five commonly used extraction protocols for isolating phlorotannins from *Cystoseira compressa*, a brown macroalga abundant in the Adriatic Sea. The protocols varied in solvent composition (methanol:water 1:1 v/v with or without pH adjustment), the inclusion of antioxidants such as ascorbic acid to prevent oxidative degradation, pre-extraction with non-polar solvents to remove interfering lipophilic compounds, and the use of solid-phase extraction (SPE) with three sorbent types (Sep-Pak C18, Oasis MCX, Oasis PRIME HLB) to improve selectivity (Lopes et al. 2018; Sardari et al. 2020; Turković et al. 2023). Phenolic content, used as a proxy for phlorotannin concentration, was quantified using the Folin-Ciocalteu assay (Torres et al. 2024).

## Results

Extraction efficiency varied significantly among the tested protocols, with total phlorotannin content ranging from $4.29 \pm 0.014$ to $8.52 \pm 0.045$ mg phloroglucinol equivalents (PGE) per g dry weight (DW). The highest yield was achieved using Oasis PRIME HLB SPE following acidified methanol:water extraction (pH 3), while methods lacking pH adjustment or antioxidant addition generally yielded lower concentrations. The results underscore the influence of pH control, antioxidant protection, and SPE sorbent selection in maximizing recovery and purity of phlorotannin-rich fractions.

## Discussion

This study shows that pH adjustment, antioxidant addition, and SPE sorbent choice strongly influence phlorotannin yield and purity from *Cystoseira compressa*. The highest yield (8.52 mg PGE $g^{-1}$ DW) was achieved with acidified methanol:water extraction (pH 3) and use of Oasis PRIME HLB SPE columns, likely due to improved compound stability and reduced oxidation. These findings align with previous research highlighting the role of controlled extraction conditions in preserving phenolic integrity and maximizing recovery. Oasis PRIME HLB exhibited superior selectivity, efficiently retaining target compounds while minimizing co-extracted impurities. By establishing an optimized protocol that maximizes yield, minimizes impurities, and preserves bioactive integrity, this study contributes to the standardization of phlorotannin extraction methods - an essential step toward reproducible research and the development of industrial-scale applications in health-promoting formulations.

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# IMPACT OF DIETARY OMEGA 3 FATTY ACIDS ON THE TRANSCRIPTOMIC AND LIPIDOMIC RESPONSES IN ATLANTIC SALMON (*Salmo salar*) FOLLOWING *IN VIVO* BACTERIAL AND VIRAL STIMULATIONS

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## Introduction

The aquaculture industry faces two major challenges, firstly infectious diseases and secondly the high cost of fish feeds. This is especially true for Atlantic salmon (*Salmo salar*). Research has demonstrated high quality, and nutritionally complete feed is linked to improved health of cultured species. Crosstalk between functional feeds and immune responses may be an effective proactive measure to reduce the need for the use of reactive disease management tools in salmon farming, especially in relation to omega-3 oils and associated phospholipids. This study examined how dietary oils impact the immune function and lipid abundance in Atlantic salmon fed three different experimental diets formulated using different oil sources (a) krill oil, (b) southern fish oil and (c) sunflower oil.

## Materials and methods

Following a feeding trial fish were immune stimulated with *Yersinia ruckeri* flagellin (bacterial stimulant), poly I:C (viral mimic) or by PBS (control), for 24 hours by intraperitoneal injection. Lymphoid tissue (head kidney) were used for RNA extraction and analysed for immune gene expression using qPCR to examine the expression of a panel of immune genes reprsenting the pro-inflammatory, anti-inflammatory, antibacterial and antiviral responses. Blood plasma samples from the same fish were analysed for eicosanoids and PUFAs using liquid chromatography-mass spectrometry (LC-MS).

## Results

In flagellin stimulated fish, mostly similar expressions of pro-inflammatory genes were observed in samples fed krill oil diet and fish oil diet. In contrast, anti-inflammatory gene expression was highest in samples fed with krill oil diet. The expression of antibacterial genes was higher in samples fed krill oil diet than fish oil and sunflower oil.

On the other hand, in Poly I:C stimulated samples, pro-inflammatory gene expressions were lowest in samples fed krill oil than fish oil and sunflower oil diets, with anti-inflammatory responses being higher in krill oil fed samples.

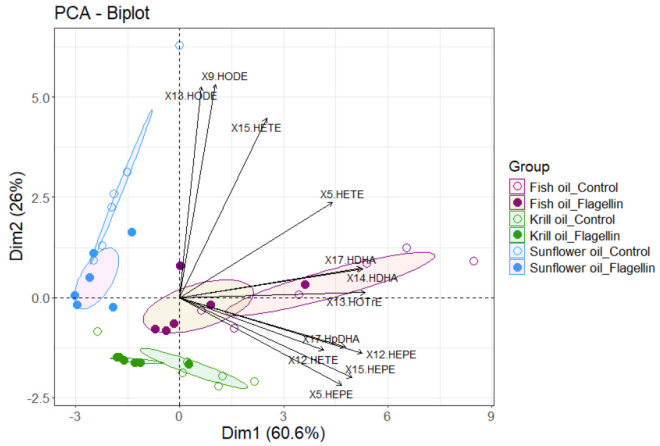


Figure 1: Principal component analysis (PCA) for blood plasma eicosanoids' abundances in control and flagellin stimulated samples

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In 24-hour post flagellin and poly I:C stimulation, the relative abundances of omega-3 fatty acids (EPA and DHA) and their eicosanoids were lower than control samples. The abundance was observed to be similar in fish oil and krill oil fed samples. In contrast, the abundance of arachidonic acid and its eicosanoids were highest in fish fed sunflower oil diet in case of both stimulations which is responsible for proinflammation (Figure 1).

**Discussion**

In blood plasma samples of fish fed krill oil and fish oil diets, EPA and DHA were used up in a similar way to modulate the immune responses against bacterial and viral stimulation.

Phospholipids (PLs) from marine-based source are rich in omega-3 fatty acids, particularly EPA and DHA. In Atlantic salmon, most of the omega-3 fatty acids come from their diet (Qian *et al*., 2020). Martinez-Rubio *et al.* (2012) compared diets rich in fish oil and krill oil both containing high PL to a diet high in rapeseed oil in Atlantic salmon. After infection with piscine reovirus (PRV) the results showed that fish on marine oil-rich diets had lower viral replication and less heart damage, likely due to a reduced inflammatory response Martinez-Rubio *et al.* (2012).

Overall, the data suggests fish fed diets rich in krill oil may reduce pro-inflammatory responses and induce anti-inflammatory genes in Atlantic salmon which may have benefits for controlling response to pathogens.

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# IMMUNOMODULATION AND ANTI-BETANODAVIRUS ACTIVITY OF THE GROWTH HORMONE RELEASING PEPTIDE-6 (GHRP-6)

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## Introduction

Nervous necrosis virus (NNV) is a betanodavirus that affects a wide range of fish species with high economic value. In the Mediterranean area, betanodaviruses belonging to the RGNNV genotype are a serious threat, especially concerning the European sea bass (*Dicentrarchus labrax*) culture. For this reason, the development of prophylactic measures is a priority for the aquaculture industry, encouraging the search of new immunostimulants, such as Growth Hormone-Releasing Peptide 6 (GHRP-6), which is a six-amino acid synthetic peptide that acts as agonist of the growth hormone secretagogue receptor. GHRP-6 has been demonstrated to have immunomodulatory activity in several fish species (Hernández *et al*., 2021; Martínez *et al*., 2022). The aim of this study was to evaluate the anti-NNV activity of GHRP-6.

## Materials and methods

The anti-NNV activity of GHRP-6 was analysed *in vitro*, on E11 cells, by the MTT-reduction assay to quantify the cell survival rate after the viral infection using different approaches (Moreno *et al*., 2023). The *in vivo* antiviral activity was assessed in juvenile European sea bass intraperitoneally injected with GHRP-6 prior to the viral infection (1 $\mu$g/g, 2 doses within 48 h). The transcription of several immuno-related genes was evaluated in head kidney and intestine 24 h after the second injection. At this time, fish were intramuscularly infected with the RGNNV isolate (2 x $10^5$ $TCID_{50}$/g), and the virus was quantified in brain by absolute qPCR (genome quantification) and the $TCID_{50}$ method (infective viral particles) 5 days after the viral challenge.

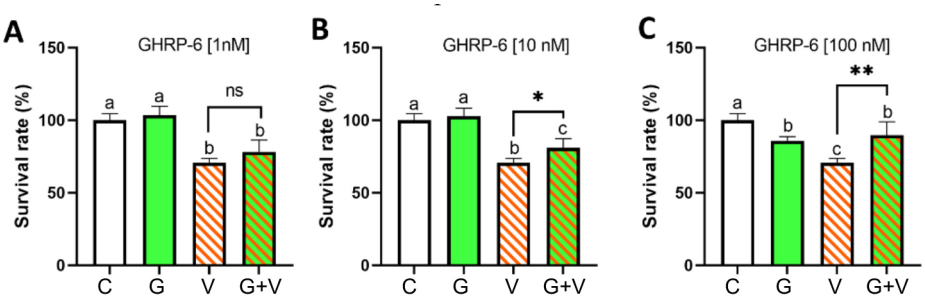


**Figure 1:** Antiviral activity of GHRP-6 against RGNNV in E11 cells inoculated at 0.1 multiplicity of infection (MOI). Survival rate of cells treated with 1 (A), 10 (B) and 100 nM GHRP-6 (C). Control cells (C, white), GHRP-6 treated cells (G, green), infected cells (V, white and red lines), GHRP-6 treated and infected cells (G+V, green and red lines). Data were analysed with the two-way ANOVA test. Different letters denote significant differences. Values of $p<0.05$ (*) or $p<0.001$ (**) were considered significant. Results are mean ± standard deviation (SD) (n = 5).

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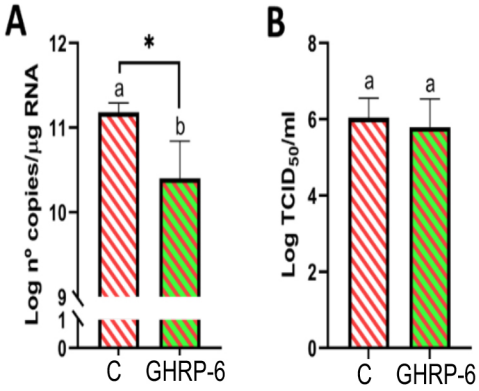


**Figure 2:** Effect of GHRP-6 on RGNNV multiplication in European sea bass brain at 5 days post-infection. (A) Absolute quantification of viral genome. (B) Infective viral particles. GHRP-6 untreated fish (white and red lines); GHRP-6 treated fish (green and red lines). Data were analysed with the two-way ANOVA test. Different letters denote significant differences; $p < 0.05$ values were considered significant (*). Results are mean ± SD (n = 5).

**Results and Discussion**

E11 cells exposed to GHRP-6 for 24 h prior to the RGNNV infection and maintained in contact with GHRP-6 during all the incubation time, were protected from RGNNV (Figure 1). In contrast, other approaches, involving cell exposure to GHRP-6 only before or after virus inoculation, did not result in protection from the viral infection.

Regarding the *in vivo* challenge, gene transcription was evaluated in head kidney and intestine and clear immunomodulatory effects were observed in GHRP-6-injected animals. Furthermore, after RGNNV infection, the number of viral genome copies in brain of GHRP-6 treated animals was significantly reduced at 5 days p.i., compared to untreated fish, although the viral titres were similar in both experimental groups (Figure 2). These results demonstrate a clear antiviral activity of GHRP-6 against RGNNV, and support further evaluation of its antiviral potential against other pathogens relevant to the aquaculture industry.

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**Acknowledgments**

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# UNCOVERING THE FUNCTIONAL SYNERGIES OF AUTOCHTHONOUS AND ALLOCHTHONOUS GUT MICROBIOTA OF FARMED SEA BREAM

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## Introduction

The gut microbiota represents a complex environment inhabited by a large number of microorganisms that play an important role in numerous physiological processes of live animals, including fish (Singh *et al*., 2025). Indeed, the microbiome present in the whole body, particularly in the gut systems of finfish and shellfish, not only contributes to digestion but also has an impact on nutrition, growth, reproduction, immune system, hereditary traits, and vulnerability of the host fish to diseases. Several factors can influence the gut microbiota structure and composition, including spatial distribution across the digestive tract and time post-feeding. Most microbiome studies target the colonizing autochthonous (resident or adherent) bacteria, as they are considered a more stable population with a more direct impact on the host. However, allochthonous (transient) bacteria may be also relevant with a recognized influence on resident bacteria and host physiology (Viver *et al*., 2023). In any case, the organization of both communities, together with their functional role, still remains far from being clear. Accordingly, this study aimed to assess how the gut autochthonous and allochthonous bacterial communities change and interact in a spatial and temporal manner in farmed fish, using gilthead sea bream as a fish model of the Mediterranean aquaculture.

## Material and methods

One-year old fish (356±12.8 g) were reared in 3,000 L flow-through tanks (IATS aquaculture infrastructure) under natural photoperiod and temperature conditions in June with water [$O_2$] levels maintained above 75% saturation. Fish were fed with a commercial diet (EFICO 3053, Biomar). At 24-h post-feeding, 10 animals were euthanized and samples of mucus and intestinal contents from the anterior and posterior intestine were collected for analysis of the autochthonous and allochthonous bacteria. Additionally, at 48-h post-feeding, mucus layer samples were taken from other 10 fish. Microbial DNA was extracted, and the 16S rRNA v1-v9 regions were sequenced (ONT MinION device) and processed with an in-house pipeline. Sequences were quality filtered and taxonomically assigned following a custom pipeline using SILVA database. A discriminant analysis (PLS-DA) of gut microbiota composition was made with EZInfo software. A Bayesian Network approach was conducted using the SAMBA tool (Moroni *et al*., 2025). Clusters of nodes connected were identified using the Leiden community detection method. Functional profile was inferred for each cluster using the KEGG database for the pathways functional annotation protocol. Statistically significant differences between abundances and functions in resident and transient bacteria were assessed through a non-parametric Kruskal Wallis test.

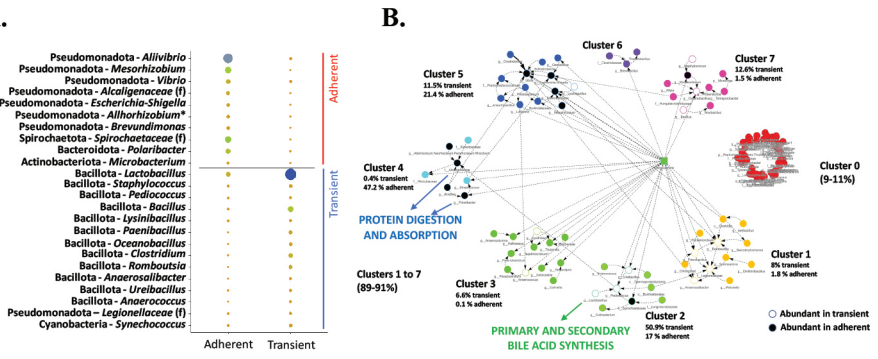


**Figure 1.** (A) Dot-plot showing genera with significantly different abundance between resident and transient bacterial communities and (B) SAMBA Bayesian network displaying clusters of interconnected bacteria and two relevant associated functions.

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**Results and discussion**

The transient bacteria presented greater values of richness (ACE), either in the anterior (286±83.3) or posterior intestine (276±40.6), in comparison to the gut adherent microbiota considered as a whole (71.2±3.29). Although the diversity indices did not reach a statistical significance, they shared a similar trend (e.g., Shannon index; transient bacteria, 2.09±0.147; resident bacteria, 1.44±0.138). Therefore, in agreement with previous studies (Sommer and Bäckhed, 2013), the mucus layer forms a more restrictive environment that supports the adherence and residence of a more stable microbial population. Besides, while the bacteria phylum Pseudomonadota was dominant in the resident community (47-80%), the Bacillota was the highest represented taxa in the transient microbiota (79-85%). Certainly, the PLS-DA displayed a significant separation between resident and transient bacteria, confirming remarkable variations in their composition. Nevertheless, no clear or smaller differences were observed within each group between anterior and posterior intestinal bacterial communities, or across different post-feeding times. Thus, we focused on the comparison of resident and transient bacteria after 24-h post-feeding, regardless of the sampled intestinal section. At the genus level, most discriminant and abundant bacteria in the adherent community belonged to the phylum Pseudomonadota (e.g., *Aliivibrio, Mesorhizobium, Vibrio,* Alcaligenaceae family*, Escherichia-Shigella, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Brevundimonas*) (Figure 1A). Conversely, in the lumen layer, abundant bacteria were mostly of the phylum Bacillota (e.g., *Lactobacillus, Staphylococcus, Pediococcus, Bacillus, Lysinibacillus, Paenibacillus, Oceanobacillus, Clostridium, Romboutsia, Anaerosalibacter, Ureibacillus, Anaerococcus*). Numerous metabolic functions (147) were associated to both microbial communities, while 54 and 44 pathways were more represented in the resident and transient bacterial communities, respectively. Accordingly, the SAMBA tool allowed to discern different bacterial clusters (Figure 1B) that reveal different links and functional synergies between specific bacteria across the two microbial gut communities. For instance, the Protein digestion and absorption appears likely linked, among others, to family Alcaligenaceae or the genus *Polaribacter* of the mucus layer. At the same time, the Primary and Secondary bile acid synthesis became specially related to some lumen bacteria taxa, including several species of the genus *Lactobacillus* (Ridlon *et al*., 2006).

**Conclusions**

Autochthonous and allochthonous bacteria form distinct communities in the gut of gilthead sea bream. Several genera inhabit both the mucus and lumen layers, but most of them are differentially represented and interconnected. Thus, mucus-associated microorganisms appear essential to preserve gut function, including digestion processes, but at the same time bacteria of the lumen layer becomes necessary for the bile acid synthesis, likely modulating the composition of the mucosal bacteria community.

**Funding**

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# MICROENCAPSULATED ESSENTIAL OILS AS NOVEL AQUAFEED SUPPLEMENTS IN RAINBOW TROUT (*Oncorhynchus mykiss*) CULTURE

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## Introduction

Aquaculture production has significantly increased over the past decade, becoming a key component of global aquatic animal production. In a highly competitive market characterized by standardized products, the establishment of a distinct product identity plays a critical role in influencing market success. Product differentiation facilitates the targeting of specific consumer segments and enables producers to achieve price premiums by offering superior quality or distinctive attributes. This strategic positioning enhances market value and supports long-term sustainability and competitiveness within the industry. On this regard, the incorporation of essential oils from aromatic herbs into aquafeeds has gained increasing attention as a natural and sustainable alternative to synthetic additives and antibiotics in aquaculture. These bioactive compounds, derived from plants such as oregano, thyme, rosemary, and basil, exhibit a range of beneficial properties including antimicrobial, antioxidant, anti-inflammatory, and immunostimulatory effects. More specifically, rosemary essential oil (REO), derived from *Rosmarinus officinalis* L. (Lamiaceae), has shown promising potential as a functional additive in aquafeeds due to its rich composition of bioactive compounds such as 1,8-cineole,  -pinene, camphor, borneol, and others. These components possess strong antioxidant, antimicrobial, and anti-inflammatory properties, which can contribute to improved fish health and performance. REO can also be used in aquafeeds to impart desirable flavor characteristics to fish, potentially enhancing the sensory qualities of the final product. Its aromatic compounds, such as 1,8-cineole and camphor, can be absorbed and metabolized by fish, subtly influencing the taste and aroma of the flesh. This flavor modulation is especially valuable in high-value aquaculture species where consumer preference for flavor and freshness is critical. However, the effectiveness of REO in flavor transfer depends on factors such as dosage, duration of feeding, fish species, and lipid content in the diet and available data are still fragmentary. Additionally, REO is prone to degradation when exposed to environmental factors such as heat, light, oxygen, and moisture, which can lead to the loss of its bioactive properties over time. To overcome these delivery/stability issues, a natural-based microencapsulation technique has been developed in the present study to enable a controlled application of REO in rainbow trout culture with the aim of assessing fish health and promoting product differentiation in the market by delivering a more appealing and consistent flavor profile to consumers.

## Materials and methods

***Rosemary essential oil isolation:*** A six-hour hydrodistillation of 1 kg dry leaves of wild *R. officinalis* (batch no C-130324-18 produced in 11/2024; Minardi & Figli S.r.l., Bagnacavallo, Ravenna, Italy) was performed to obtain ROE. The EO was recovered through a Clevenger-type glass apparatus. For the chemical characterization, the EO was diluted 1:100 in *n*-hexane and then analysed with an Agilent 8890 gas chromatograph (GC) equipped with a single quadrupole 5977B mass spectrometer (Santa Clara, California, USA). The system was equipped with an autosampler PAL RTC120 (CTC Analytics AG, Zwingen). The chromatograms obtained were studied by using the MSD ChemStation software from Agilent and the NIST Mass Spectral Search Program; linear retention indices (RIs) and analytical standards available in the authors' laboratory were also used for peak assignment. ***Microencapsulation:*** The REO was microencapsulated by STM Aquatrade S.r.l. (Castelraimondo, Macerata, Italy) utilizing a novel technology named Co.M.E. (Coating MadeEasy) based on natural molecules. Microcapsules are able to stick to any aquafeed and deliver their content to the fish.  The provision of 1.5 g of microcapsules corresponded to a net transfer of 25 ppm of the REO to the feed. ***Experimental design:*** 120 adult rainbow trout (400 ± 30 g) were divided into two experimental groups in triplicate: Control and REO group fed a control diet and a control diet supplemented with microencapsulated REO, respectively. Fish were daily fed with experimental diets (0.8% of the body weight) for 90 days. At the end of the trial fish were euthanized to evaluate zootechnical performances and welfare (growth performances; histological analyses of liver and gut; molecular analyses of fish stress and immune responses as well as the effectiveness of REO in flavor transfer in fish fillets).

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**Results and Discussion**

The chemical composition of rosemary essential oil (REO), as determined by gas chromatography-mass spectrometry (GC-MS), was predominantly characterized by 1,8-cineole (47%), camphor (19%), α-pinene (10%), borneol (5%), α-terpineol (4%), *p*-cymene (2%), and limonene (1%), with other constituents occurring at relative percentages below 1%. The microencapsulation technique developed in this study effectively preserved the integrity of REO and enabled its targeted delivery through aquafeed. No significant adverse effects on fish growth performance or welfare were observed following REO supplementation. Furthermore, evidence of flavor transfer from REO to fish tissue was demonstrated, indicating its potential application in enhancing the sensory quality of aquaculture products.

Belfiore E.A. PhD is funded by “PR Marche Fondo Sociale Europeo Plus 2021/2027”

# WORLD VETERINARY EDUCATION IN PRODUCTION ANIMAL HEALTH (WVEPAH) A WOAH COLLABORATING CENTRE FOR ADVANCED TRAINING IN LIVESTOCK ANIMAL HEALTH "CONTINUING EDUCATION MUST DRIVE FOOD SECURITY FOR ALL"

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**The situation:**
By 2050, more efficient animal production will be required. The animal production sector faces intense pressure to increase the supply of high-quality, safer food while preserving natural resources and environment.

Human activities have a direct impact on the environment, and governments, civil society and consumers expect the animal production sector to adopt the best management procedures and mitigation strategies to reduce its impact.

The intensification of animal production has exposed animals to more diseases and is contributing to the development of multi resistant bacteria (AMR), threatening both animal and human health. The interconnections between animals and people have stressed the importance of the One Health approach in addressing the global rise of antimicrobial resistance (AMR).

Therefore, WVEPAH thinks the holistic approach towards increasing the sustainability of animal production is essential for global food security.

While intergovernmental institutions are proposing regulations and standards to improve the sustainability of animal production and fair trade, veterinarians, animal production specialists, and producers need concrete and practical support and guidance.

WVEPAH is training and certifying animal production professionals working closely with producers. These experts work in the private or public sectors, in international institutions or companies acting locally, regionally or globally.

- We strive to provide these experts with the latest knowledge and tools to address health and animal production management in a holistic approach, the only approach for the sustainability of animal production.
- We believe that our experts are valuable partners to help producers:
- Attain the best possible outcome for their production systems, improve animal production, health and welfare in a sustainable manner and produce healthier and safer foods.
- Understand that prevention is the best way to deliver sustainable results.
- Implement health management and biosecurity plans to prevent or detect diseases and emergency situations early, mitigate their impact and reduce the risks of antimicrobial resistance.
- Become more resilient to drastic changes with thorough understanding of optimal health and performance parameters on their farms and regions
- Adopt international standards to make trade smoother and more predictable.

WVEPAH brings a concrete One Health teaching and learning approach to animal production and health for all. Through training and certification, we empower our experts to serve as the ultimate resources for intergovernmental organizations, driving the changes needed to sustainably improve the world's food supply and leave no one behind.

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**WVEPAH programs: A series of 80-hour modules are available**

The programs target professionals with a veterinary degree or a diploma in agronomic science in related fields.

- The Module I:" Fundamentals" and WOAH Regulations: Teaches the systemic approach of production systems and how to evaluate if the system is threatened: Management of performances, Nutrition and Feeding, Environment, Biosecurity & Health: hygiene, surveillance, AMR management, diseases, Physiology, epidemiology. This module gives professionals strong bases and technical know-how.
- The Module II – "Specialised" delivers teaching focused on specific productions and geographical areas and challenges.

These modules have a hybrid format: On-line followed by a face-to-face 5-day event at one of WVEPAH's host veterinary faculties all around the world, combining lectures and practical sessions, clinical cases review and discussion. Our Course Masters are internationally recognized specialists in their respective fields and participants greatly benefit from their broad practical experience.

**Certifications and Diploma worldwide validated by WOAH**

All participants at WVEPAH modules receive a certification of attendance. Participants who successfully completed Modules I and II and submitted 25 clinical cases or a series of farm audits are awarded the Professional Certificate in Animal Health: Poultry, Aquaculture or Swine.

Production by the University of Montreal and validated worldwide by WOAH. This post-graduate degree confers de facto to their recipients the status of International Expert in Aquaculture health management.

**Achievements and Future objectives**

We have several hundred certified experts around the World. WVEPAH will be a main institution recognized for providing up-to- date, global knowledge, training experts to ensure Food security for all, humans and animals alike. The next Aquaculture training sessions for 2026 will be held in France and Singapore.

# MICROBIOLOGICAL QUALITY AND SHELF LIFE EXTENSION OF REFRIGERATED NILE TILAPIA (*Oreochromis niloticus*) FILLETS SUPPLEMENTED WITH *Artemisia annua*, ARTEMISININ, AND ARTEMETHER

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## 1. Introduction

Fish are vulnerable to microbiological and chemical deterioration due its intrinsec factors such as high-water activity, neutral pH and high content of polyunsaturated fatty acids (PUFAs) (Santos et al. 2017). Food quality is assessed by microbiological analyzes and postmortem changes that alter sensory and physicochemical characteristics (Aracati et al., 2022). During storage, they undergo lipid oxidation, affecting their sensory characteristics and nutritional quality (Rodrigues et al., 2025). Nile tilapia (*Oreochromis niloticus*) is highly perishable, and preserving its quality during storage is critical for reducing food waste and meeting nutritional demands. This study explores a sustainable approach using dietary supplementation with *Artemisia annua*, artemisinin, and artemether, natural compounds with antioxidant and antimicrobial properties.

## 2. Material and Methods

A total of 160 tilapia were randomly assigned to four treatments: control (no additive), 1% *A. annua* powder, artemisinin (9.6 mg/kg feed), or artemether (9.6 mg/kg feed). After 30 days of feeding, 320 fillets were collected and stored under refrigeration at 4 °C. Samples were collected and analyzed at four different time points: immediately after slaughter (day 0), and then on days 7, 15, and 30. For each treatment group,10 for microbiological evaluations, which included counts of mesophilic and psychrotrophic bacteria, molds and yeasts, sulfite-reducing Clostridium, Enterobacteriaceae.

## 3. Results

The MPN results for thermotolerant coliforms showed no significant differences ($p \geq 0.05$) among treatments. However, total coliform counts were significantly reduced in fillets from fish treated with artemether after 30 days of refrigeration. Mesophilic and psychrotrophic counts at 7 and 30 days of storage were significantly lower ($p < 0.05$) in treated groups, particularly artemether, compared to controls. Although all groups showed significant microbial growth ($p < 0.05$) over the 30-day shelf life, the increase was more pronounced in the control group. Enterobacteria counts were significantly lower in fish treated with artemether or artemisinin after 7 days of refrigeration; however, counts increased over time in all groups.

No significant differences were found between treatments for mold and yeast counts, although all groups showed an increase after 15 days of refrigeration. Sulfite-reducing bacteria counts showed no significant differences ($p \geq 0.05$) between treatments, with no growth observed. Coagulase-positive staphylococci counts were significantly higher ($p < 0.05$) in control fillets at 15 and 30 days compared to treated groups, with increases over time in both control and Artemisia annua-treated fish.

## 4. Conclusion

The results of microbiological analyzes demonstrate the benefical participation of Artemether, artemisinin and *Artemisia annua* in the shelf-life of tilapia fillets stored under refrigeration. The results of this study revealed that these dietary supplementations were effective in maintaining fish quality and extending shelf life, in addition to having the potential to be used as a food additive due to bioactive compounds.

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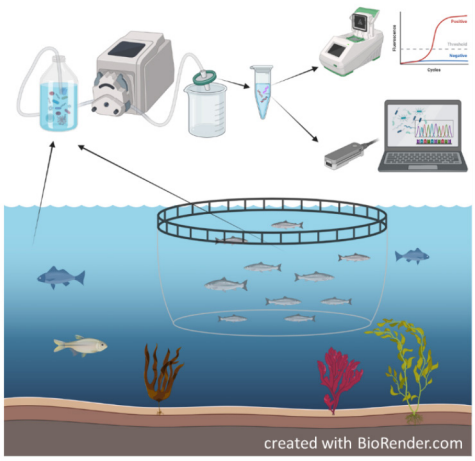


**Acknowledgments**

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# OPTIMIZED METHODS FOR WATER-BASED PATHOGEN DETECTION IN AQUACULTURE SYSTEMS

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## Introduction

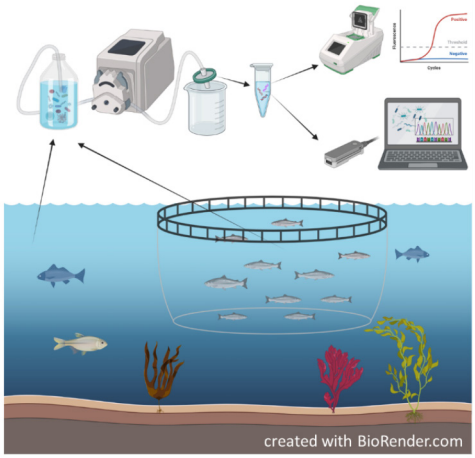
Specific and sensitive tools for detecting and monitoring pathogens in the aquatic environment can be crucial for preventing the spread of infectious agents in aquaculture facilities. Monitoring waterborne pathogens through water filtration and subsequent detection using target-specific (RT-) qPCRs and/or sequencing is an approach that can reduce or even replace the need to euthanize fish for monitoring purposes in aquaculture, allowing earlier biosecurity- and disease control measures.

## Material and methods

We first performed an *in vitro* work where we tested various filtration- and nucleic acid extraction methods for selected pathogens prior to target-specific (RT-) qPCRs to simplify and facilitate on-site water filtration without compromising detection probability. This was tested in combination with different filtration rates, lysis buffers, and filters for freshwater and seawater spiked with relevant salmon pathogens. The suitability and feasibility of the methodology was then tested in the field, where a fish group was followed over time from the hatchery to offshore Atlantic salmon aquaculture facilities until slaughter. Additionally, the third generation sequencing approach (MinION Oxford Nanopore Technologies) was used to correlate shotgun metagenomics sequencing for microbiome analyses to events in the production cycle.

## Results

The results showed that a "sandwich" filtration method combining two different filters allows for the detection of various pathogens with a single filtration step and a single nucleic acid extraction step, followed by (RT-) qPCRs. This reduces both the costs and time required for analysis. Moreover, we showed that this methodology is feasible and effective under field conditions. This means that aquaculture facilities could implement these methods on-site, ensuring timely and accurate pathogen monitoring. Additional shotgun metagenomics sequencing for microbiome analyses with the MinION allowed a microbial screening technique which would lead to future real-time acquisition and analysis of data in the field. This dual qPCR-sequencing approach will provide a unique system to gain knowledge on the impact of real-time monitoring to detect various pathogenic specimens (e.g., viruses, bacteria, parasites).



# CLOSING THE LOOP: IMTA INGREDIENTS AS A SUSTAINABLE PATHWAY IN FISH FEED

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**Introduction**

Aquaculture is one of the fastest-growing food production sectors, but it also faces increasing scrutiny due to its environmental impact—particularly the high carbon footprint associated with aquafeed production. As the demand for seafood rises, there is an urgent need to reduce the industry's environmental burden by identifying low-carbon or carbon-neutral alternatives to conventional feed ingredients. One promising yet underutilized solution lies in the use of materials derived from Integrated Multi-Trophic Aquaculture (IMTA) systems (Rusco et al., 2024). These systems produce nutrient-rich by-products that can be repurposed as sustainable feed ingredients, turning waste into value while closing resource loops (Masi et al., 2024). The utilization of IMTA-derived ingredients not only helps lower the carbon footprint of aquafeed but also supports a broader transition toward a more circular, resilient, and eco-friendly aquaculture industry. Among the most promising IMTA-derived candidates for sustainable feed are species such as polychaetes and seaweed, which offer both nutritional value and carbon mitigation potential:

- Polychaetes (*Sabella spallanzanii*): A carbon-sinking polychaetes species cultivated in IMTA on long lines (Arduini et al., 2023).
- Seaweed (*Ulva lactuca*.): commonly used as a "biofilter" in IMTA and reach in protein and lipids, widely utilized as aquafeed ingredients (Shpigel et al., 2017):

This study investigates the potential of incorporating these IMTA produced ingredients into the diet of juvenile seabass (*Dicentrarchus labrax*), aiming to supports the development of circular food systems that can enhance the resilience and economic viability of aquaculture operations.

**Materials and Methods**

This trial was conducted at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences, University of Bologna (Cesenatico, Italy). Juvenile seabass (*Dicentrarchus labrax*; initial average weight 5 ± 2 g) were sourced from Panittica Italia and acclimated for two weeks. Fish (n= 100/tank) were randomly stocked into nine 450 L square tanks (three replicates per dietary treatment) and reared for ~30 days. Tanks operated within a closed recirculating aquaculture system (RAS) equipped with mechanical sand filtration, ultraviolet lights, and a biofiltration as described by Parma et al. (2023). Water parameters were maintained at 24 ± 1.0 °C, 25 ± 5.0 g/L salinity and oxygen levels at 8.0 ± 1.0 mg/L using a liquid oxygen system. Feed was produced at the University of Almeria and the ingredients have been directly collected by an experimental IMTA plan located at Marina Grande (Taranto). Fish were fed 4 different pelleted isoproteic and isolipidic diets:

- Ctrl: 20% FM + 5% shrimp/krill meal
- Diet 1: 20% FM + 5% Sabella meal
- Diet 2: 20% FM + 5% Sabella meal + 5% Ulva meal
- Diet 3: 20% FM + 5% Sabella meal + 10% Ulva meal

Feeding was performed to satiation with a 10% surplus twice daily (8:30 and 16:30) six days per week. At the beginning and end of the experiment, all fish are anesthetized or euthanized with tricaine methanesulfonate (MS-222). Growth performance and whole-body and dietary proximate analyses parameters are calculated. Gene expression related to digestion (*amyl, lip* and *pept*), appetite (*pomc a, ghrl, cart*), growth (*gh, igf1*, oxidative stress (*sod* and *cat*), immune related genes (*il1β* and *il10*) and physiological stress (*hsp70* and *hsp90*) are analyzed to evaluate the nutritional and physiological response to the different diets. All experimental procedures were approved by the Ethical Scientific Committee for Animal Experimentation of the University of Bologna (ID 113/2020-PR), following European Directive 2010/63/EU.

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**Figure 1:** Specimen of *a*) *Sabella spallanzanii and b) seaweed Ulva lactuca from IMTA in Marina Grande of Taranto;*

**Results and Discussion**

Preliminary results suggest that the inclusion of *Sabella spallanzanii* and *Ulva lactuca* in the diet for juvenile seabass do not have negative effects on growth performance and biochemical composition of fish. This is because both Sabella meal And Ulva meal are rich in high-quality proteins containing essential amino acids required for growth and development, and these proteins are highly digestible. Furthermore, an increase in feed intake (FI) and the expression of genes related to appetite regulation (*ghrl* and *pomca*) is observed due to the known high palatability of *Sabella spp*. Additionally, *Sabella* spp. as well as Ulva contain bioactive compounds such as peptides and polysaccharides that have immune-boosting properties, as observed from the upregulated expression of *il10* and reduced *hsp70*. However, large-scale production and harvesting of *Sabella* spp. may involve high costs, potentially impacting the feasibility of its inclusion in commercial aquafeeds. Despite these challenges, when properly managed, dried *Sabella* meal in combination with Ulva meal can promote growth, enhance feed palatability, and boost immunity due to their superior protein quality, beneficial lipids, and rich mineral content.

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# MULTI-USE LOW-TROPHIC AQUACULTURE, OFFSHORE WIND, OR FISH FARMING: EXPANDING the OLAMUR PROJECT FROM THE BALTIC/NORTH SEA TO THE BLACK SEA, THE MEDITERRANIAN AND THE ATLANTIC

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The urgent need for more efficient use of marine area, and for European energy and food security, has highlighted the potential for multi-use, i.e. the use of area for more than one purpose. The recent expansion of offshore wind power has emphasized conflicts among different stakeholders. At the same time, aquaculture of low-trophic organisms, such as bivalves or seaweeds appears an attractive way to increase biomass production. Both activities contribute to urgent European needs, for energy and food security. Bivalves can feed on microalgae and particles, and seaweeds utilize nutrients, such as nitrates, phosphates and $CO_2$. In the OLAMUR project, we are developing initiatives for multi-use involving *low-trophic aquaculture* (LTA) in the Baltic and North Seas. Now, the project is expanding with five Associated regions across different European seas.

*Low Trophic Aquaculture* can improve water quality and ecosystem services. Furthermore, it is an attractive way to produce large quantities of biomass in an eco-friendly way, with low footprint, combined with potential for environmental mitigation of over-fertilization and excess carbon. It adds to a circular bioeconomy. At the same time, it contributes to improve Europe´s supply of biomass, thereby its food security.

The addition of Associated Regions (to the OLAMUR project, implies that five additional case studies will be added to the project. All contracts have now been signed, and the five regions are operational.

The Associated Regions, added to the project in February 2025, are operating in different European waters:

1. Black Sea: Kavarna Municipality, Bulgaria
2. Meditteranean: Adana Metropolitan Municipality, Adana, Turkey
3. Atlantic: Midc Páirc na Mara Dac, Galway, Ireland
4. Atlantic : Município de Viana do Castelo, Portugal
5. Atlantic: The Spanish National Research Council, Galicia Spain

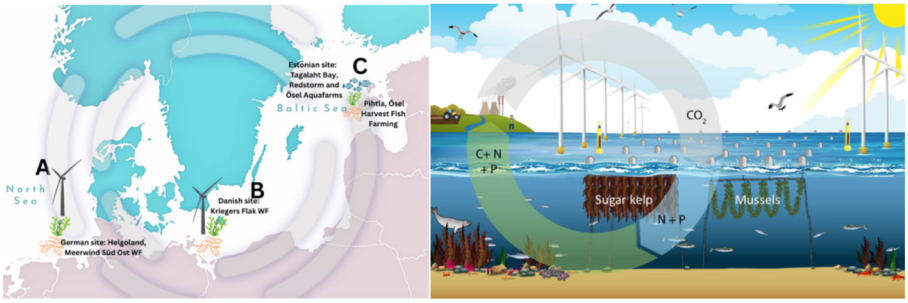


*FIGURE 1 (Left) The three case studies of OLAMUR, in the North Sea (A) and Baltic Sea (B). In case A (Germany) and B (Denmark), low trophic aquaculture of seaweeds (kelp and* Ulva*) and mussels (*Mytilus edulis*) are taking place within the areas of ongoing large scale wind farm operation. In case study C (Estonia), low trophic culture (Ulva and mussels) are carried out adjacent to a rainbow trout farm.*

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These projects in the Associated Regions are highly different with regards to TRL level. Common to them all is an emphasis on stakeholder mapping and analysis. Offshore wind are in place in some, but not all, Regions. Furthermore, the aquaculture activities are highly different, and the potential is different, due to variations in environmental and societal conditions. The Associated Regions will be integrated in OLAMUR project throughout the remaining life cycle of the project, and provide reports together with the main project and in collaboration with it.

An extensive Data Management Plan ensures transparent and standardized data capture from all parts of the project, as well as its interoperability towards European Marine data infrastructures (EMODnet and Copernicus Marine) for project data legacy (Scotto et al. 2023)

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# URGENT NEED FOR MARINE SPATIAL PLANNING OF THE NORWEGIAN EEZ EMPHASIZED BY EXPANSION OF OFFSHORE AQUACULTURE, OFFSHORE WIND, AND OTHER INDUSTRIES

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Europe´s need for increased energy and food security has been emphasized by the Ukraine war, and the weakening of the Trans-Atlantic alliance. Europe imports 70% of its seafood, and even with the inclusion of Norway, Iceland and the Faroes the continent is heavily dependent on import from outside Europe. To a large degree this is also the situation with energy, where the import of oil and gas from Russia must be replaced by non-European sources. Energy security and food security may be viewed as equally critical in a European as well as a global geopolitical context. Also, to counteract global warming, to adapt to a changing environment, and to facilitate $CO_2$ emissions reduction, there is an urgent need to upscale renewable energy production (Maar et al. 2023).

Pressure is increasing for more extensive utilization of marine areas. This is especially true in the coastal zone (Bergh et al. 2023), but is becoming more evident in offshore environments. This requires careful study of the impact each industry has on its environment and on local communities, considering the cumulative effects from all industries combined. Careful consideration of uncertainties will be crucial to reduce the considerable trust gap among ocean stakeholders, such as fishermen, shipping industry, environmentalists, aquaculturists, and the renewable as well as fossil energy industries.

It should be emphasized that the Norwegian EEZ already is heavily exploited by fisheries and the oil/gas industry. As Figure 2 shows, especially the North Sea, but also the continental shelf towards the Norwegian Sea, and the shallower Barents Sea have a range of oil/gas installations, and in conclusion, the expansion of "new" offshore industries are further emphasizing the need for Marine Spatial Planning (MSP) in the Norwegian EEZ (Pettersen et al. 2024). Careful consideration on the need for marine protected areas (MPAs) should in particular take into account important ecosystem services, as spawning areas and specially vulnerable areas, coral reefs and migration areas for fish juveniles.

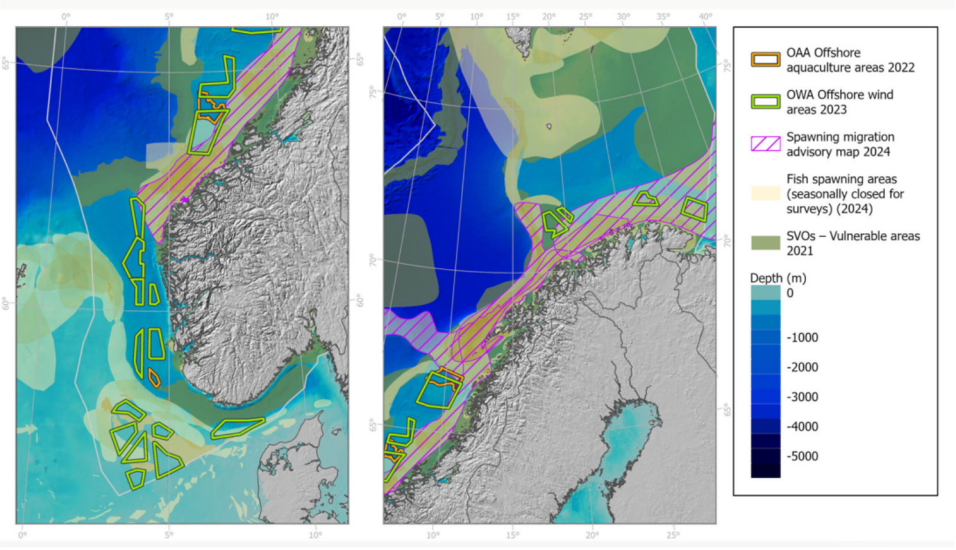


*FIGURE 1 Suggested area for offshore wind and offshore aquaculture, by the Norwegian Ministry for Energy and the Ministry for Industry, trade and Fisheries, respectively. here, the suggested areas are superimposed on the SVO (Specially vulnerable areas) and the fish spawning areas and migration areas for juvenile fish, as mapped by the Institute of Marine Research.*

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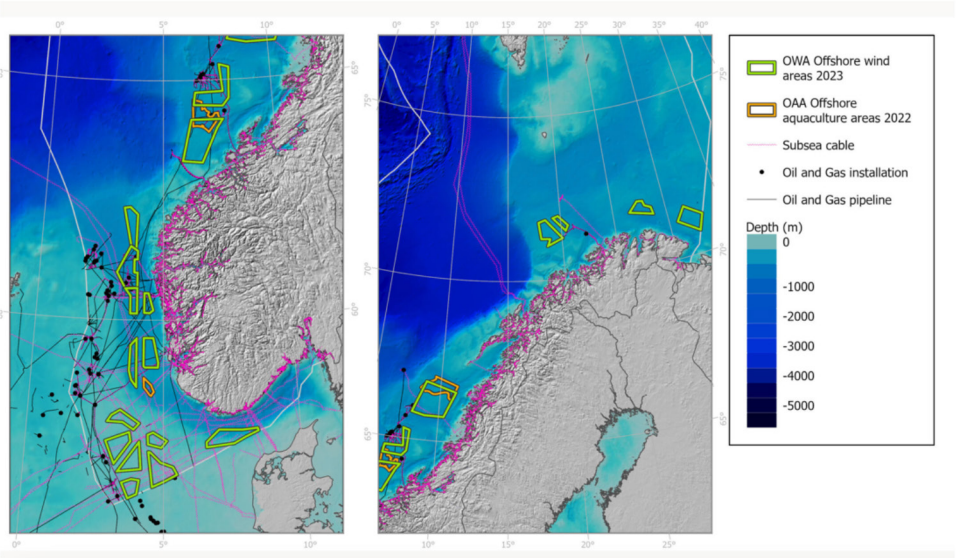


*FIGURE 2. Oil/gas installations, pipelines and electric and fiberoptic cables surrounding Norway, together with the suggested areas for offshore wind and offshore aquaculture*

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# DOES NUTRITIONAL PROGRAMMING INFLUENCE ATLANTIC SALMON (*Salmo salar*) RESPONSE TO MICROALGAE IN FEEDS?

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## Introduction

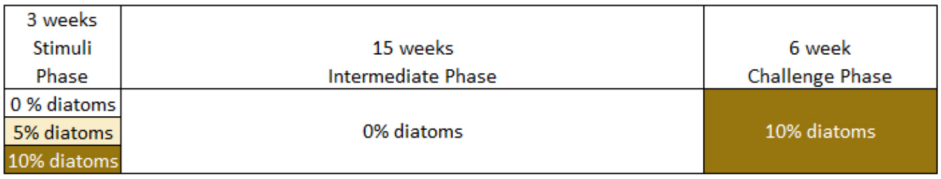
There is a need for more sustainable feed ingredients for farmed animals and Norway has a future goal that "all feed for farmed fish and livestock shall come from sustainable sources and contribute to reducing greenhouse gas emissions in the food system". Microalgae is a promising candidate as a feed resource and in this project a marine diatom that grows fast, contains high amounts of DHA and EPA and all essential amino acids (Artamonova et al., 2017; Jónasdóttir, 2019)Coscinodiscus concinnus, Porosira glacialis, and Chaetoceros socialis, were cultivated in nutrient replete growth medium and harvested in the exponential growth phase. Cultivation took place at temperatures similar to ambient late spring bloom temperature (7 °C will be studied as a feed ingredient for Atlantic salmon (Salmo salar).

Nutritional programming is a field of research that in mammalian studies have focused on suboptimal early nutrition and predisposition of metabolic diseases later in life, while in fish there are documented effects on growth, survival and nutrient metabolism (Clarkson et al., 2017; Hou and Fuiman, 2020)such as permanent growth retardation and impairment of neural development and key metabolic pathways. This phenomenon, termed nutritional programming or metabolic programming, is beginning to be studied in fishes. Despite important differences in maternal nutrient delivery and developmental processes between mammals and fishes, early nutrition of fishes from both endogenous (maternally derived. Some of these documented effects of different live feeds in marine species (Imsland et al., 2006; Koedijk et al., 2010; Øie et al., 2017)a second fed enriched rotifers (RotMG could be explained by altered metabolic pathways and/or epigenetic regulation of gene expression, but there is a lack of studies addressing nutritional programming and long term effects. Previous studies in Atlantic salmon have shown that salmon can be adapted to plant-based ingredients by including these in the starter feed. The feed consumed by the salmon in its early weeks causes long-term changes in gene expression, potentially enabling the fish to better utilize new types of feed ingredients later in production (Clarkson et al., 2017; Sahlmann et al., 2015; Tawfik et al., 2024)fry were fed either a marine-based diet (Diet Mstimulus, 80% fishmeal (FM.

To evaluate the effects of early dietary exposure to diatoms on nutritional programming of Atlantic salmon fry, a 24-week freshwater feeding trial will be conducted, commencing at the onset of exogenous feeding (yolk sac absorption). The trial consists of three distinct phases also shown in Figure 1:

1. **Stimulus Phase (Weeks 0–3):** During the first three weeks, salmon fry will be fed one of three experimental diets containing 0%, 5%, or 10% diatom inclusion. This phase aims to initiate potential long-term changes in gene expression associated with early nutritional stimuli.
2. **Intermediate Phase (Weeks 4–18):** All groups will then transition to a common control diet without diatoms, allowing for assessment of the persistence of early nutritional effects without continued exposure.
3. **Challenge Phase (Weeks 19–24):** In the final six weeks, all groups will be challenged with a diet containing 10% diatoms to assess whether early dietary exposure influences the fish's ability to utilize diatom-based feed ingredients later in development.

*Figure 1: Overview of feeding phases in the 24-week nutritional programming study*

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This stepwise approach represents a classical model for investigating nutritional programming.

**Material and methods**

Atlantic salmon fry (400-day degrees post hatch) was provided from Aquagen and transported to our facility at SINTEF in Trondheim, Norway. The trial started in 100-liter tanks with flow through fresh water at 8 °C and increased to 11 °C over 7 days with 3 experimental groups and 4 replicas resulting in 12 tanks, with 24h light. Three experimental diets with 0%, 5% and 10% microalgae were produced by Skretting, Norway. In the stimulus phase of 3 weeks, the experimental feeds were fed from the onset of exogenous feeding. In the following intermediate phase, a commercial diet (Skretting) was given and in the last 6 weeks of the trial, during the challenge phase a diet with 10% algae was provided (produced by Skretting). The pellet size of the diets was adapted to the size of the fish using advice from Skretting as a guideline. When the average weight of the fish was about 7 g the fish was pit tagged (Biomark, USA) and transferred to 2 tanks of 4,5 $m^3$ tanks where all groups were mixed until the end of the trial. Fish was sampled after 3 weeks and before and after the challenge phase. Analysis performed was growth, microbiome in gut, gut histology, transcriptome analysis of gut and lipidomic analysis of muscle. At the end of the trial critical swimming capacity was tested in a swimming respirometer and the filet quality was tested with hyperspectral imaging.

All procedures complied with the Norwegian Animal Welfare Act of 20 December 1974, No. 73 (Sections 20–22, amended 19 June 2009). The experiment was approved by the Norwegian Food Safety Authority (FOTS ID 31092).

**Results and Discussion**

Results and discussion will be provided later as the trial is not finalised.

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# ECONOMIC VIABILITY OF SEAWEED AND SEA CUCUMBER TANK CULTURE IN IMTA WITH OYSTERS OR MILKFISH ON ZANZIBAR, TANZANIA

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New and sustainable aquaculture systems need to be developed in order to meet the ever so increasing demand for food. In developing countries especially, there is a great need for sustainable yet low-cost aquaculture to provide local farmers with food and income. This study looked at the co-culture of seaweed and sea cucumbers under different conditions, including integrated multitrophic aquaculture (IMTA) systems with oyster and recirculating aquaculture systems (RAS) with milkfish, in Zanzibar, Tanzania. Seaweed production was especially aggravated by the ice-ice disease, resulting in specific growth rates (SGRs) between 1.32 and -1.96 % $d^{-1}$. Nitrogen content in the seaweed thallus and inorganic nutrients in the water showed good results, thus demonstrating the potential of seaweed to be used as a biofilter. An economic analysis showed the economic viability of IMTA systems, co-culturing *Kappaphycus alvarezii*, *Holothuria scabra* and *Pinctada margaritifera*, achieving a maximum benefit-cost ratio (BCR) of 1.61 over one year. Furthermore, interviews with local farmers on Zanzibar showed their willingness to accept new aquaculture systems. However, the farmers also aired a lot of constraints, such as a lack of infrastructure, funds and expertise.

# LARGE-SCALE CRYOPRESERVATION AFFECTS SPERM CHARACTERISTICS AND HATCHING RATE BUT NOT GROWTH, MALFORMATION AND SURVIVAL RATE IN CHUB (*Squalius cephalus*) LARVAE

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The objective of this study was to investigate the growth, malformation and survival rate in chub (*Squalius cephalus*) larvae originating from large-scale cryopreserved sperm. Sperm was cryopreserved in accordance with a protocol (modified extender designed for northern pike, 10% methanol, 1:1, 4 mL cryotube, Styrofoam box) previously developed in our laboratory. Firstly, the motility and different kinetic parameters (fresh, control, cryopreserved) of sperm (control and cryopreserved) used for propagation, and the hatching rate were evaluated. Secondly, the growth (average body weight, total length), malformation and survival rate were assessed in a 12-day rearing experiment (0, 8, 12 days post-hatching - DPH) by the larvae originating from control and cryopreserved sperm.

A significantly lower motility (MOT), progressive motility (pMOT), and linearity (LIN) were recorded in the cryopreserved group compared to the fresh and control groups. The distance average path (DAP), curvilinear velocity (VCL), straight line velocity (VSL), beat cross frequency (BCF) showed significant differences in all three groups. The control group (96±1%) exhibited a significantly higher hatching rate in comparison with the cryopreserved group (70±14%). Sperm cryopreservation had no major negative effect on the growth (except only in total length at 0 DPH), malformation and survival rate of chub larvae. Extending the study period to encompass the complete pre-nursing or grow-out periods in future research would be beneficial.

The research was funded by the GINOP-2.2.1-18-2020-00026 grant. Furthermore, our work was also co-financed by the project 2022-1.2.6-TÉT-IPARI-TR-2022-00002 within the framework of the National Research, Development and Innovation Fund announced by the Ministry of Culture and Innovation. This research was also funded by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2021, National Defense and Security sub-programme (TKP2021-NVA-22). This work was supported by the Research Excellence Programme and the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences. Our experiments were funded by the EKÖP-MATE/2024/25/K (EKÖP-24-V/MATE-6) university research scholarship Programme of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund. The study was supported by the project "Support for talent management programmes in colleges" (NTP-SZKOLL-23-0043), commissioned by the Ministry of Culture and Innovation and funded by the National Cultural Fund of Hungary.

# EFFECTS OF BOAT NOISE ON THE BEHAVIOUR AND PHYSIOLOGY OF THE EUROPEAN SEA BASS (*Dicentrarchus labrax*): A FOCUS ON FAMILIARITY AND EXPOSURE PATTERNS

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**Introduction**

It is widely recognized that the marine environment is impacted by noise pollution generated by human activities. Maritime traffic is the primary source of diffuse broadband noise in the marine environment, including aquaculture systems, and can have negative effects on marine organisms. Exposure to ship noise pollution can elicit behavioral responses and increase physiological stress in fish and appears to mask acoustic communication with effects on foraging, navigation and reproduction (Popper & Hawkins, 2019). The boat is an essential tool for sea-cage rearing, but it can itself be a frequent and long-lasting stressful factor for the fish that cannot escape. The mode of noise exposure, i.e. gradual or immediate, is also an aspect to be considered in order to have the necessary information to design or apply noise mitigation measures in sea farming but there are few studies that provide information on those aspects.

By combining field monitoring and controlled exposure experiments, the present study aims at assessing the acute noise effects on European seabass stress response and behaviour and the potential effects related to the familiarity of the boat and the sound exposure pattern.

**Materials and Methods**

90 adult European seabass were exposed to boat noise in experimental tanks in groups of five, after elastomer tagging and acclimation. From the acoustic recordings collected at sea, two 30-minute boat noise tracks were selected: (A) the noise from a boat considered "familiar" to the animals, as it was the same vessel that regularly visited the sea cages for the management; and (B) the noise from a boat with similar acoustic characteristics but not experimented by the animals so representing an "unfamiliar" sound to them.

Each tank were exposed for one hour to one of the following 5 noise conditions: background noise of fish farm's origin (BG), familiar boat noise played in a progressive (PF) or sudden pattern (SF), unfamiliar boat noise played in a progressive (PU) or sudden pattern (SU).

After exposure, fish blood and tissues were collected following anaesthetic overdose (MS222, Sandoz) and samples were stored at -20°C until analysis. Cortisol, as main stress hormone, was measured by Radioimmunoassay (RIA) in different matrices (plasma, mucus, fin, and scales) to assess the fish stress response avoiding sampling effects and potential fish chronic stress status before the exposure (Bertotto *et al.,* 2010). Haematocrit as well as plasmatic glucose, lactate and heat shock protein levels (HSP70) were assessed, as secondary stress response indicators, by means of an automatic analyser (BIONSEN, EKF Diagnostics) and a commercial ELISA kit (Cusabio). Physiological indicators were compared across treatments through Bonferroni correction test ($P<0.05$).
Behavioral data was collected on videos, in five non-consecutive 5-min intervals, one before and four during the exposure. DeepLabCut, a machine learning for the markless pose estimation using a deep neural network with supervised learning, was trained to track fish and collect data related to the movements, specifically the swimming speed. Behavioural data were analysed separately for each noise exposure via Generalized linear mixed model, with sampling interval as a fixed factor and the tank as a random factor.

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**Results and Discussion**

Overall, physiological data showed mild variation across different noise exposure conditions and no stress response. Plasmatic cortisol showed higher levels in the progressive and sudden unfamiliar noise groups (PU and SU) than in the background group (BG, $P<0.005$) and no differences with the familiar boat noise groups, both progressive and sudden (PF and SF, $P>0.05$). The latter were not different from the background group and overall, the levels were well below the basal ones indicating no stress response (concentration <88 ng/ml; Samaras, 2023). Also, the cortisol in fins and scales, indicators of previous and chronic state of stress, showed baseline values albeit with some differences between groups ($P<0.001$; Bertotto *et al.,* 2010; Samaras *et al.,* 2021). Hematocrit, glucose, lactate and HSP70 levels confirmed the absence of variations among groups ($P>0.05$) and non-stress conditions.

The analysis of movement revealed a significant increase in speed following the onset of sound exposure ($P < 0.05$), regardless of the condition, indicating a sudden behavioral response. When considering specific conditions, individuals in the BG group appeared to recover from the initial startle response, exhibiting a gradual reduction in speed over the subsequent hour. This recovery pattern was not observed in the other groups. In the SU group, fish initially responded to the background sound but showed a further significant increase in speed upon the onset of the sudden sound stimulus ($P < 0.05$).

**Conclusions**

Plasma levels of cortisol and swimming speed seem to identify unfamiliarity and sudden exposure as the factors that most affect the stress response in European sea bass exposed to boat noise. Basal levels found in all physiological indicators analysed indicate a lack of physiological response to stress despite significant behavioural alteration related to swimming speed. This suggests a disconnect between immediate behavioural responses and typical markers of physiological stress in the context of noise exposure, which requires further investigation.

**Acknowledgement**

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# ENHANCING GROWTH AND FEED EFFICIENCY IN FRESHWATER ATLANTIC SALMON THROUGH PARTIAL FISHMEAL REPLACEMENT WITH DEFATTED BLACK SOLDIER FLY MEAL

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## Introduction

Over the past decade, aquaculture and particularly Atlantic salmon farming have undergone a significant nutritional transition, shifting from a fishmeal-based diet to relying on plant-based ingredients[1]. While this shift has contributed to reduced dependency on marine resources, it has also raised concerns relating to land use, health, gut function, and fillet quality[2,3]. Additionally, the volatility in prices and availability of both marine and land-based raw materials is intensifying the search for novel, sustainable, and functional feed ingredients. Insect meal has emerged as a promising candidate, considered a well-suited nutrient source for salmonids, which naturally consume insects in their riverine habitats[4]. The objective of the study was to determine whether 50% of fish meal content in freshwater phase Atlantic salmon diets could be replaced with partially defatted Black Soldier Fly Larvae (BSFL) meal (corresponding to 10% inclusion of BSFL meal in the diet), and its impact on key performance indicators such as growth performance and feed efficiency.

## Materials and Methods

Defatted Black Soldier Fly Larvae (BSFL) meal from Innovafeed replaced 50% of fishmeal in the test diet (corresponding to a 10% inclusion), which was otherwise iso-proteic and iso-energetic to a fishmeal/SPC control. 100 juvenile Atlantic salmon (17.5g initial weight) per tank (n=4 replicates/diet) were fed to satiation 4 times daily for 8 weeks. Feed intake, growth (initial and final weight, SGR, FCR), mortality, and VSI (5 fish/tank at termination) were measured. All parameters were assessed for normality and homogeneity of variance prior to statistical analysis. Subsequently, comparisons were performed using a Student's *t*-test.

## Results

After eight weeks of study, the highest feed intake was observed in the insect-meal fed group (+ 4%, $p > 0.05$), which also had a higher final weight (+ 5%, $p < 0.05$) compared to fish fed the fishmeal based control. This higher final weight translated into a significantly higher specific growth rate (SGR) for the diet with 10% insect meal (1.44 vs. 1.35, $p = 0.0136$). Interestingly, despite a higher feed intake, there was a trend for the feed conversion ratio (FCR) of the test feed to be better than the control feed (0.91 vs. 0.96, $p = 0.0716$ ). Regarding fish mortality, physiologic state and health , no differences were measured between the control and test groups. More specifically, mortality was below 1% in both groups, and viscero somatic index (VSI) was similar in the control and test groups (14.7 for the control diet and 14.9 for the test diet, with no statistical significance).

## Discussion

The results obtained were particularly interesting in regards to previous studies. The improved growth performance (higher SGR, trend for better FCR) observed in our freshwater salmon trial with a 10% inclusion rate, corresponding to 50% replacement of fishmeal by insect meal offers a contrast to other studies such as Belgit et al.'s, which reported no significant effects at a 60% BSFL inclusion replacing both fishmeal and SPC[5]. As highlighted by Weththasinghe et al.'s meta-analysis, the BSFL inclusion rate and the type and percentage of protein replaced are critical[6]. Additionally, the absence of visceral fat accumulation in our study, unlike at higher inclusion rates (Belgit et al.[5]), further underscores the importance of the inclusion level.

## Conclusion

These findings indicate that a moderate level of insect meal inclusion can effectively enhance growth and feed utilization in juvenile Atlantic salmon reared in freshwater. This overperformance should be considered in the economic evaluation of incorporating insect meal as a sustainable and performance-enhancing ingredient in salmonid aquaculture.

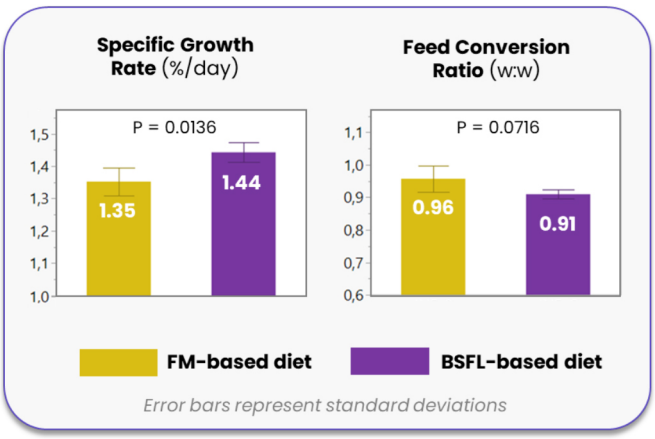


Fig 1. Specific Growth rate and Feed conversion ratio of the FM-based diet (control feed) and the BSFL-based diet (test feed) after 8 weeks of feeding at the freshwater phase.

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# PRELIMINARY STUDY ON BIOFILM IN DIFFERENT COUPON MATRICES INSERTED INTO RAS (RECIRCULATING AQUACULTURE SYSTEM) DURING AN EXPERIMENTAL TRIAL WITH EUROPEAN SEA BASS *Dicentrarchus labrax*

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**Introduction**

The function and productivity of RASs (Recirculating Aquaculture Systems) are influenced by abiotic and biotic parameters, particularly water quality and microbiology. Bacterial communities in RAS environment encompass both free-living bacteria in the water column and bacteria colonizing tank surfaces, fish and biofilters (biofilm).

Bacterial biofilms pose a significant challenge in aquaculture, serving as persistent sources of bacterial infections for reared species. Understanding on which material in such closed environment, potentially pathogenic bacteria preferably attach to form biofilms is of fundamental importance with the aim to carry out proper prevention strategies and to control possible sources of disease within the system (Schoina et al., 2022).

In this preliminary study, coupons made by different materials were used as support to simulate the main components of the RAS: fibreglass, steel and PVC. The coupons were placed in a tank with European sea bass (*Dicentrarchus labrax*) during a nutritional trial and subjected to the main qualitative and quantitative analyses of the composition of the cultivable bacteria that inhabit the biofilm formed in the coupons.

**Materials and methods**

The test was conducted in a RAS of 12 tanks of 500 litres each fed with previously disinfected natural sea water. Each tank contained 40 European sea bass (*D. labrax*) weighing approximately 30 grams fed with a commercial diet for juvenile sea bass.

Series of coupons simulating the materials used for RAS were placed inside the tanks in this order from the surface: PVC, fiberglass and steel. They had circular shape and the same diameter (22 mm) and were tied together with a nylon thread. The test was done with 3 sampling time: T0 with the coupons inserted in the water for a week without fish, T1 with the coupons inserted after 3 weeks with fish and T2 after 6 weeks. For each sampling a set of coupons was coloured with crystal violet to verify the presence/absence of biofilm. Another set underwent coupon biofilm disruption and dilution plating in TSA + 1.5% NaCl agar and TCBS agar. After carrying out the CFU/coupon counts, the most representative colonies were identified by Maldi TOF (Bruker).

**Results**

Staining with crystal violet indicates that the biofilm is formed and this happened preferably in fiberglass and steel (Fig. 2).

The bacterial counts appeared non-linear but confirm a higher bacterial load in fiberglass compared to other materials. The most representative colonies were identified by Maldi-TOF and, in addition to environmental *Vibrio* spp., potentially fish pathogenic *Vibrio harveyi* and *Photobacterium damselae damselae* were identified.

Further investigations are necessary to analyse the composition of the biofilm also with metagenomics.

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Fig. 1. Tank with coupons

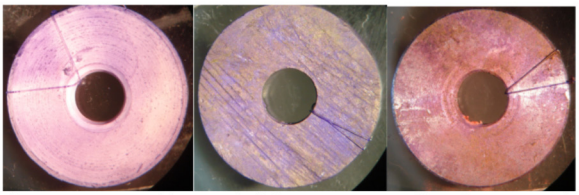

Fig. 2. Coupons with crystal violet. A: PVC; B: fiberglass; C: steel.

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# TOXICITY ASSESSMENT OF BIOBASED POLYURETHANE NANOPLASTICS ON THE MARINE MICROALGAE *Tisochrysis lutea* AND *Oltmannsiellopsis unicellularis*

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**Introduction**

Plastics provide numerous benefits to many sectors but their persistence in the environment has led to widespread pollution, and as a consecuence, microplastics (MPs) and nanoplastics (NPs) represent a serious threat to ecosystems. Bioplastics, such as waterborne polyurethanes (WBPUs), are being developed as more sustainable alternatives. However, the environmental impact of these new materials, especially in their nano-scale size, remain largely unknown. Interactions between microalgae and plastics are expected in the ocean; however, toxicity patterns are not well established. It has already been reported that differences in the coverage of microalgae species can affect microalgae sensitivity to xenobiotics. Therefore, further research on the impact of plastics including bioplastics such as WBPUs, on microalgae strains with varying structural characteristics is required.

**Materials and methods**

This study aimed to assess the toxicity of three types of biobased polyurethane (PU) nanoparticles: (1) partially biobased PUs or WBPUs, (2) WBPUs synthesized using the organic solvent N-methyl-2-pyrrolidone (WBPU-NMP) and (3) WBPUs partially derived from chemically recycled PET (WBPU-BHET). Types 1 and 3 were tested with (WBPU-3CNC and WBPU-BHET-CNC, respectively) and without nanocellulose (WBPU and WBPU-BHET, respectively) incorporated as an additive. Thus, the nanocellulose by itself (3CNC) was also tested. Additionally, for comparative purposes, a commercial folmulation of WBPU nanoparticles (WBPU Covestro Impraperm DL3746 XP) was also tested. As a result, the ecotoxicological effects of seven different compounds were tested on two marine microalgae species, the scales bearing haptophyte *Tisochrysis lutea* and the naked chlorophyte *Oltmannsiellopsis unicellularis*. Growth inhibition tests (OECD guideline nº 201) were run on microalgae exposed to a concentration range (0.01 to 100 mg/L) of each WBPU type and to the equivalent concentrations of 3CNC (3 $10^{-4}$ to 3 mg/L). Since OECD nº201 criteria were confirmed in a shorter period of time for *T. lutea* than for *O. unicellularis* (48 h for *T. lutea* and 72 h for *O. unicellularis*), production of reactive oxygen species (ROS) and maximum quantum yield of photosystem II ($F_v/F_m$) were studied on *T. lutea* exposed to selected concentrations (0.01, 1 and 100 mg/L) of WBPU nanoparticles and to the equivalent concentrations of 3CNC (0.0003, 0.03 and 3 mg/L).

**Results**

Growth rate of both microalgae was only reduced after exposure to the highest concentration of some of the WBPUs tested: WBPU-BHET altered the growth of both microalgae while WBPU-BHET-CNC only altered the growth of *T. lutea* and WBPU-NMP did the same to *O. unicellularis*. Therefore, the two microalgae species exhibited different sensitivity to WBPU NPs which could have been conditioned by the formation of different size heteroaggregates in the marine microalgae exposure media. ROS production was increased in comparison to controls when *T. lutea* microalgae were exposed to all concentrations of WBPU COVESTRO and to 0.01 and 1 mg/L of WBPU-3CNC as well as to the equivalent concentrations of 3CNC. On the contrary, ROS production was reduced when microalgae were exposed to 1 and 100 mg/L of WBPU-NMP. No effects were recorded in $F_v/F_m$.

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**Discussion**

Selection of microalgae species that show different structural/morphological characteristics seems crucial for the ecotoxicological assessment of bioplastics such as WBPUs, since different organisms seem to respond differently to the same treatments. The observed variation in sensitivity between *T. lutea* and *O. unicellularis* highlights the importance of considering the composition of nanomaterials as well as species-specific traits, such as cell wall composition, surface charge and size, which can influence interactions with nanoparticle aggregates, especially in marine environments. The significant alteration of ROS production depending on the WBPU type and concentration, indicates that subcellular stress responses can occur even in the absence of growth inhibition or photosynthetic impairment while the lack of effects on Fv/Fm across all treatments suggests that oxidative stress may not necessarily translate into functional damage to the photosynthetic apparatus, at least over such short exposure periods.

**Conclusion**

WBPUs are considered a sustainable alternative to conventional PUs, however, both physicochemical characterization of the materials tested and the biological responses of ecologically relevant species must be integrated into further research to better assess the toxicity of bioplastics, such as WBPUs, and their ecological risks

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# OPTIMIZATION OF GROWTH CONDITIONS OF *Chlorella sorokiniana* (CHLOROPHYCEAE) USING A BREWERY WASTEWATER

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**Introduction**

Beer is the most widely consumed alcoholic beverage globally, with billions of litres of beer being produced each year. The beer production process is water-intensive and generates large quantities of brewery wastewater (BWW), which must be treated before being discharged into wastewater treatment plants. This poses an economic challenge for small beer-producing companies, which characterize the beer sector in the Basque Country. In this context, microalgae cultivation using BWW has emerged as an alternative to traditional treatment methods, as it enables removing nutrients from the BWW, while also producing value-added microalgae biomass, which currently has various commercial applications.

This study aimed to explore the potential use of a BWW from a small craft beer company, Laugar Brewery (Gordexola, Biscay), as a culture medium for microalgae.

**Materials and Methods**

Based on the literature, the chlorophyceans *Chlorella sorokiniana* and *Acutodesmus obliquus* were selected as candidate species for the study. Two strains of each nominal species were acquired from the Banco Español de Algas for an initial screening. Additionally, a fast-growing non-identified green algal strain isolated from our laboratory was included in the study. The five strains were phylogenetically characterized using 18S rDNA as a marker. Subsequently, in order to select one of the strains for growth assays in BWW, all microalgal growth was evaluated using mineral water enriched with F/2 nutrients as culture medium (48-well microplates; 23 ºC; continuous illumination 100 µmol photons $m^{-2}$ $s^{-1}$). Strain BEA0003, confirmed to be *Chlorella sorokiniana,* was selected for subsequent optimization assays.

Three independent batches of BWW were collected from the first cleaning step of the mash tank. The BWW was stored at two temperature conditions (room temperature and 4 º C) for four weeks ($T_0$ - $T_4$) with samples collected weekly. The samples were filtered (0.22 µm) and subjected to one of the three treatments: sterilization by ultraviolet (UV) light exposure, sterilization by autoclaving or not post-filtration sterilization. Filtered samples from $T_0$ and $T_4$ stored at room temperature without sterilization were analyzed for total organic carbon (TOC), total nitrogen (TN) and pH.

For growth optimization, the effects of several variables on the maximum specific growth rate ($\mu_{max}$) were tested: initial cell concentration, different concentrations of nutrient supplementation ($NaNO_3$, $NH_4Cl$, $NaH_2PO_4$ and F/2 micronutrients), dilution of the BBW with mineral water, and the storage conditions and sterilization conditions of the BBW as previously described. Microalgal growth was monitored via chlorophyll fluorescence using a microplate reader. Finally, under optimized conditions (undiluted BBW supplemented with 110 µM $NaNO_3$), a nutrient removal experiment was conducted using BWW from $T_4$ stored at room temperature without post-filtration sterilization as culture medium. The culture was grown for 7 days, with an initial cell concentration of 5 $10^5$ cells/mL and nutrient removal was assessed by measuring TOC and TN concentrations before and after cultivation.

**Results and Discussion**

Initial identification based on DNA sequences revealed a mismatch between the species label and the actual identity of one strain, highlighting the importance of DNA-based identification when working with microalgae. Strain BEA0003 confirmed as *Chlorella sorokiniana* was selected for further experiments due to its high $\mu_{max}$ ($1.99 \pm 0.08$ $d^{-1}$) under the tested conditions and its ability to grow in suspension.

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The analysis of the chemical composition of three different BWW batches revealed an appropriate pH (7.3-7.7) but significant variability in TOC and TN along with low nitrogen concentrations. This suggested an imbalance in C/N and N/P ratios, which might limit microalgae growth. This was supported by the observed improvement in growth with nitrogen supplementation, particularly with nitrate. However, no significant differences were found among BWW dilutions or between different nitrate concentrations, with 110 µM identified as the recommended supplementation level. Phosphorus or micronutrient supplementation did not enhance microalgal growth, indicating that this BBW is not limited on these nutrients. Therefore, the findings suggest that the BWW can be used undiluted if nitrogen (110 µM $NaNO_3$) is supplemented, without the need for additional phosphorus or micronutrients. Moreover, BWW stored at room temperature for four weeks ($T_4$), without refrigeration or post-filtration sterilization, significantly enhanced microalgae growth compared to freshly collected BBW ($T_0$). The $\mu_{max}$ values varied significantly across different BBW batches, indicating that variability in chemical composition influenced its suitability as a culture medium for microalgae. Nevertheless, under the defined culture conditions (BWW stored at room temperature, without post-filtration sterilization and supplemented with nitrate), high $\mu_{max}$ values ranging between 1.24 and 1.50 $d^{-1}$ were achieved. Nutrient removal was also successful, with TOC removal between 23.2 and 45.3 % and TN removal between 69.6 and 86.9 %, resulting in a final effluent considered safe for disposal. These results demonstrate that cultivating the chlorophycean *Chlorella sorokiniana* in this BWW is a viable strategy for the valorization and reuse of these wastes, while simultaneously contributing to reduced microalgal biomass production costs and water consumption.

This work was funded by the Basque Government through grants to the consolidated research groups IT1743-22 and IT1723-22, and the Fundación HAZI Fundazioa.

# UNLOCKING THE NUTRITIONAL VALUE OF *Arthrospira platensis* INDUSTRIAL BY-PRODUCT: PROTEIN EXTRACTION AND IN VITRO DIGESTIBILITY

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## Introduction

*Arthrospira platensis* (spirulina) is a cyanobacterium renowned for its rich content of bioactive compounds including proteins, lipids, carbohydrates and minerals. Among its commercially valuable products, C-phycocyanin (a blue phycobiliprotein with antioxidant and anti-inflammatory properties) stands out. However, the industrial extraction of phycocyanin generates a residual by-product biomass that, despite its high protein content, remains largely underutilised. Within a biorefinery framework, this by-product represents a promising resource for developing high-value functional ingredients. This study explores ultrasound (US) and high-pressure homogenisation (HPH) as cell disruption techniques to enhance protein recovery from the residual biomass after phycobiliprotein extraction. Furthermore, the nutritional potential of all *A. platensis* fractions (including raw and by-product residual biomass and the resulting protein concentrates) was assessed. Protein digestibility and bioaccessibility were assessed via *in vitro* gastrointestinal digestion following the standardised INFOGEST protocol, while protein quality was determined using the Digestible Indispensable Amino Acid Ratio (DIAAR), providing a comprehensive assessment of their nutritional value and potential application in food systems.

## Materials and Methods

Proteins were solubilized from *A. platensis* by-product under alkaline conditions. Ultrasound (US) and high-pressure homogenisation (HPH) were applied as cell disruption techniques to enhance the extraction yield, followed by acid precipitation to recover solubilised proteins. The physicochemical properties of the resulting protein concentrates were characterised. Samples underwent *in vitro* digestion according to the INFOGEST protocol to assess digestibility. Protein digestibility was quantified by total nitrogen analysis, free amine measurement via the o-phthalaldehyde (OPA) assay and total amino acid (TAA) determination. Finally, DIAAR values were calculated for each sample to evaluate the quality of the digested proteins.

## Results

Protein extraction yields from the by product biomass reached 49.1% with US and 61.0% with HPH, showing superior efficiency with HPH. Protein content isolate yields were 60.7% (US) and 63.5% (HPH), varying slightly with the disruption method applied.

Raw *A. platensis* biomass exhibited an average protein digestibility of 91%, which decreased to 78% in the HPH-derived protein concentrate, indicating that processing intensity may reduce digestibility. Despite this reduction, nutritional quality remained high; DIAAR values for most essential amino acids exceeded 100% in all samples, highlighting their excellent nutritional potential. Lysine was the limiting amino acid in protein concentrates, with DIAAS values dropping to 68%, below the FAO-recommended 75% threshold. These findings suggest that mild cell disruption techniques can effectively recover proteins from *A. platensis* by-product, whose favorable nutritional profiles support their potential incorporation into food formulations. This valorization strategy contributes to waste reduction and aligns with circular economy principles.

# FUTURE LINES OF STUDY ON BLUEFIN TUNA (*Thunnus thynnus*)

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## Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*, ABFT) is one of the most valuable species in aquaculture, but farmed populations experience high and unexplained mortality rates that threaten production sustainability (Ortega, 2015). This mortality remains a major bottleneck to scaling up production, with particularly high and variable losses during the larval and early juvenile phases (Betancor *et al*., 2019). The underlying causes of this low survival are multifactorial, potentially involving nutritional condition, quality and availability of live prey, environmental factors, handling stress, collisions with tank walls, sudden unexplained deaths, and developmental abnormalities, some of which may be associated with physiological alterations and changes in the gut microbiome. Two ongoing studies led by the team at the land-based tuna facility at Mazarrón (ICTS-ICAR, IEO-CSIC, www.icar.ieo.es) address these aspects: one focused on cardiac health and histology, and another on gut microbiota composition.

## Study 1 – Cardiac health

Optimal cardiac health is essential for the sustained swimming and high metabolism of pelagic predators, yet in aquaculture, stressors can impair heart structure and function, reducing performance and survival (Engdal *et al*., 2024). While such effects are well documented in Atlantic salmon, whether they occur in farmed Atlantic bluefin tuna is unknown. Despite its high metabolic demands and reliance on sustained cardiac output (Blanco *et al*., 2020), detailed descriptions of ABFT heart morphology are scarce, with most information extrapolated from other tunas such as yellowfin (*Thunnus albacares*) and Pacific bluefin (*T. orientalis*), which have characterised ventricular anatomy, myocardium composition, and coronary circulation in relation to swimming performance and thermal tolerance (Korsmeyer *et al*., 1997; Shiels *et al*., 2015).

The present study investigates the role of cardiac health in unexplained mortalities in ABFT aquaculture. By analysing ventricular morphology and histology (H&E, Masson's Trichrome) in individuals from wild, fattened, and fully cultured origins, and spanning larval to adult stages, we aim to identify structural or fibrotic patterns associated with captivity. Comparative evaluation of wild and farmed fish will provide baseline descriptions for the species and assess whether observed differences could contribute to mortality events.

## Study 2 – Gut microbiota

The gut microbiome supports nutrient uptake, immunity, and disease resistance (Ringø *et al*., 2016). In aquaculture, diet, density, and environment can reshape communities, especially during early life when larvae acquire microbes from water and prey and immunity is immature, influencing growth and pathogen resilience. As fish mature, microbiota adapt to diet and habitat.

Research on tuna microbiomes is scarce, with existing studies - mainly on wild juveniles and adults of yellowfin (*T. albacares*), bigeye (*T. obesus*), and skipjack (*K. pelamis*) - showing species-, growth-, and environment-driven shifts in gut communities (Gadoin *et al*., 2021; Zou *et al*., 2024; Huang *et al*., 2025). However, larval stages and farmed individuals remain largely unstudied, and no work has compared wild and cultured ABFT across life stages. This gap reflects a broader absence of ontogenetic data in teleost gut microbiome research. This study characterises the intestinal microbiota of *Thunnus thynnus* across life stages and rearing conditions to address a major knowledge gap. We monitored microbial succession in larvae (4 - 25 dph) under different feeding regimes (rotifers, *Artemia*, yolk sac larvae) and sampled juveniles and adults from cultured and wild origins. DNA-based sequencing of anterior and posterior intestinal sections will reveal how age, diet, and captivity shape microbial diversity and composition, identifying patterns potentially linked to health, performance, and survival in aquaculture.

**Outlook**

Both studies address key knowledge gaps in ABFT biology and have the potential to reveal physiological and microbial indicators linked to mortality in culture. The outcomes are expected to guide strategies for improving husbandry practices, reducing losses, and enhancing the sustainability of bluefin tuna aquaculture.

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# LINKING BEHAVIOUR DISRUPTION TO EPIGENETICS, TRANSCRIPTOMICS AND OXIDATIVE STRESS IN JUVENILE SEABASS (*Dicentrarchus labrax*) EXPOSED TO CADMIUM AND CIPROFLOXACIN

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## Introduction

Potential hazardous contaminants have been identified in aquatic environments coming from different anthropogenic sources. Cadmium (Cd) is a trace element commonly found in aquatic systems as a contaminant. It has both natural and anthropogenic sources, such as industrial discharges and agricultural runoff [1]. Cd effects on neurodevelopment and behavior are well established for mammals, while more comprehensive studies of its neurotoxicity on aquatic organisms such as fish, are still missing [1]. On the other hand, Ciprofloxacin (CIP) is a fluoroquinolone antibiotic frequently found in water bodies due to its incomplete metabolism in mammals and its inefficient removal by water treatment facilities [2]. CIP has been associated with genotoxic effects and increased production of reactive oxygen species (ROS), posing aquatic life at risk [3]. Moreover, its growing presence in aquatic environments may alter microbial communities, potentially compromising the gut health of fish and interfering with neuronal functions through the microbiota-gut-brain axis.

This study aimed to elucidate the neurotoxicity of Cd and CIP in juvenile seabass (*Dicentrarchus labrax*) upon chronic dietary exposure (21 days).

## Methods

One month prior to the start of the experiment, fish were randomly distributed into ten 600 L tanks (n = 30 fish per tank). During this acclimation period, all fish were fed a non-treated diet. Experimental diets were prepared following the alcohol evaporation method adapted for the European sea bass [4]. Treatments were as follows: Control (non-treated diet), CIPL (ciprofloxacin 10 mg•kg$^{-1}$ food), CIPH ciprofloxacin (10 mg•kg$^{-1}$ food), CDL (cadmium 100 mg•kg$^{-1}$ food), CDH (cadmium 10 mg•kg$^{-1}$ food). Experimental diets were administered for 21 days, each in duplicate (i.e., two tanks per condition), under the same natural photoperiod and temperature conditions. During the experiment, all groups were fed *ad-libitum*, twice a day. The estimated daily contaminant intake per treatment was CIPH, 0.182 mg ciprofloxacin, CIPL, 0.018 mg ciprofloxacin; CDH, 1.992 mg cadmium, CDL, 0.177 mg cadmium. At the end of the experiment brain samples were taken to undertake a multidimensional approach comprising epigenetics (DNA methylation by EM-seq), transcriptomics (RNA-seq), antioxidant defences and oxidative damage in the brain, neurotransmission (assessed by acetylcholinesterase - AChE) and behaviour. Biochemical endpoints related to the antioxidant defences were assessed including superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx) activities, as well as total glutathione (GSHt) content, while oxidative damage was evaluated by lipid peroxidation (LPO).

## Results and discussion

Exposure to both Cd and CIP resulted in a decrease in brain glutathione levels, potentially making brain cells more susceptible to oxidative stress. Interestingly, a significant reduction in LPO was observed in CIPL fish, whereas a slight increase in LPO was detected in CDH fish. In contrast, SOD activity increased in the brains of CDH-exposed fish, with no significant changes observed across CIP treatments. Increased AChE activity was recorded in the brain of fish exposed to both concentrations of Cd and CIP. This suggests potential disruptions in neurotransmission, as AChE has a crucial role in breaking down the neurotransmitter acetylcholine (ACh). Excessive AChE may lead to reduced ACh levels, which can impair neural signaling.

Both Cd and CIP exposure significantly altered motor behaviour. Overall, a significant reduction in basal locomotor activity

was observed in fish exposed to both Cd and CIP, regardless of their concentrations. This effect was probably related to the observed increase in brain AChE activity. Moreover, a tendency towards increased cohesion was recorded, reaching statistical significance in the CDH group. In addition, all exposed fish showed an increase in the appearance of freezing episodes, typically associated with anxiety-like responses and depressive states.

In summary, the observed decrease in swimming activity, changes in social behaviour and altered exploratory and decision-making behaviours, accompanied by reduced brain glutathione levels and disrupted neurotransmission, clearly indicate a neurobehavioral impact of environmentally relevant concentrations of Cd and CIP. These results suggest that both contaminants may compromise the antioxidant defence system, eventually leading to oxidative stress, and interfere with neural function and behaviour, potentially affecting fish survival and ecological fitness.

Multi-omic analyses are currently underway to further elucidate changes in transcriptomic and DNA methylation profiles, aiming to link molecular alterations with the observed effects on antioxidant defences, neurotransmission, and behavior. This study highlights the neurotoxicity potential of Cd and CIP in fish and points at the need for further investigation into their ecological consequences.

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# DIETARY SUPPLEMENTATION WITH A NATURAL ANTIOXIDANT MODULATES METABOLIC RESPONSES AND HEPATOCYTE STRUCTURE IN EUROPEAN SEABASS (*Dicentrarchus labrax*) UNDER CHRONIC DENSITY STRESS

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## Introduction

The high stocking densities are normally used to reduce production costs in the aquaculture industry. However, if it is not appropriate for the reared species, it can become a stressor that often triggers chronic responses and negatively affects feed intake, fish metabolism and, at the end, growth. Thus, finding methods to reduce stress and improve welfare status is essential to increase stocking densities during aquaculture production. The use of nutraceutics is presented as a solution to improve fish welfare in aquaculture activity (Hu et al., 2025). This study assesses the effectiveness of a phytogenic additive with antioxidant properties (AquaNeox, TECNOVIT-FARMFaes Ltd.) in the diet of the European seabass (*Dicentrarchus labrax*) juveniles reared at different stocking conditions.

## Materials and methods

Seabass juveniles (n = 480; $w_i = 105 \pm 30$ g) were placed into 200 L-tanks and distributed in four different experimental groups in triplicate, defined as the combination of feeding (with or without AquaNeox supplementation at 0.05 %) and stocking densities. Initial low density (LD) was defined as 5 kg/m$^3$ and high density (HD) as 37 kg/m$^3$. Fish were maintained under controlled temperature and photoperiod and fed to apparent satiation for two months under these rearing conditions. Periodic biometric samplings were performed to determine growth parameters and adjust feeding. At the end of experimental time target tissues were sampled to assess (blood and liver) physiological status.

## Results and discussion

Inclusion of the nutraceutical AquaNeox did not negatively affect fish growth. Histomorphometry analysis revealed a significant reduction in hepatocyte area in fish fed with the additive (Figure 1A), suggesting a reduction in cytoplasmatic content (volume) of hepatocytes. Conversely, hepatic values of metabolites such as glucose, glycogen and cholesterol did not differ significantly between the experimental groups. However, liver triglyceride levels increased significantly in the AquaNeox groups (under LD and HD conditions) ($p < 0.05$; Figure 1B). One possible explanation for this response is that the antioxidant capacity of AquaNeox stimulated liver lipid accumulation due to peripheral lipolysis and subsequent increased uptake of non-esterified fatty acids by liver cells and the esterification of the excess into triglycerides (Du et al., 2008).

Additionally, AquaNeox contributed to maintaining plasma glucose levels under HD conditions, whereas in control group decreased significantly ($p < 0.05$; Figure 2A). Plasma cholesterol and triglycerides remained stable in fish fed with AquaNeox and reared under HD conditions, showing no significant differences compared to LD group. However, plasma lactate and proteins values decreased significantly in HD group respect to LD group ($p < 0.05$; Figures 2B and 2C), regardless of whether the additive was used or not. Moreover, LD group fed AquaNeox enhanced significantly proteins levels compared to control group ($p < 0.05$; Figures 2C), possibly reflecting a protective effect against oxidative stress.

## Conclusions

The inclusion of AquaNeox modulates hepatic metabolism by reducing hepatocyte cytoplasmic volume and increasing liver triglyceride storage, which is possibly due to altered lipid handling pathways. The additive also helped to maintain plasma glucose levels under HD condition and to increase proteins values under LD condition. Overall, these results suggest a stabilising effect of nutraceutical AquaNeox on energy metabolism. This metabolic adjustment may contribute to improved physiological resilience under intensive farming conditions.

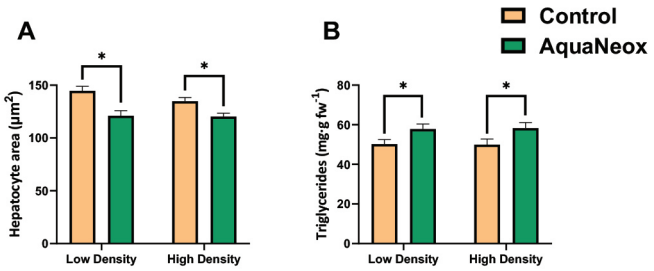


**Figure 1.** Liver parameters related to lipid metabolism in *D. labrax* juveniles fed AquaNeox under different stocking densities. Hepatocyte area (A), and hepatic triglycerides levels (B). Asterisks indicate significant differences between diets within the same stocking density ($p < 0.05$).

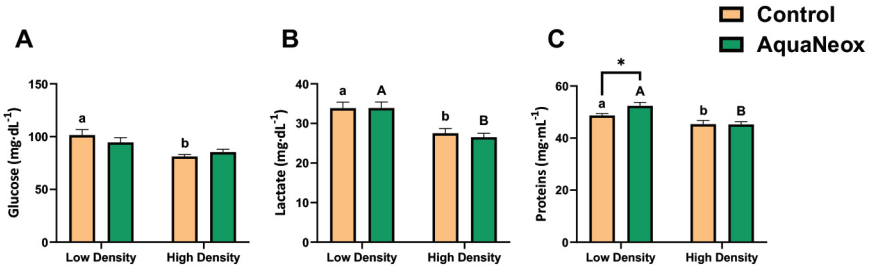


**Figure 2.** Plasma parameters in *D. labrax* juveniles fed AquaNeox under different stocking densities. Glucose levels (A), lactate levels (B), and proteins levels (C). Different capital letters represent significant differences between stocking densities within the AquaNeox diet ($p < 0.05$). Different lowercase letters represent significant differences between stocking densities within the Control diet ($p < 0.05$). Asterisks indicate significant differences between diets within the same stocking density ($p < 0.05$).

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# STRESS MITIGATION IN EUROPEAN SEABASS (*Dicentrarchus labrax*) USING A PHYTOGENIC DIETARY ADDITIVE: MODULATION OF GROWTH, METABOLIC RESPONSES, AND HEPATOCYTES' STRUCTURE

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**Introduction**

Intensification of aquaculture practices often involves increasing stocking densities, a strategy that can affect negatively animal welfare. Chronic exposure to crowding stress may lead to metabolic alterations, impaired growth, and immune suppression, ultimately reducing production performance. Functional feed additives with stress-modulating properties are gaining attention as a tool to mitigate physiological impact of intensive farming (Ciji and Akhtar, 2021). This study evaluates the efficacy of a phytogenic additive with calming properties (RELAQUAX, Bedson España S.L.) in the diet of European seabass (*Dicentrarchus labrax*) reared under different stocking density conditions.

**Materials and methods**

A total of four experimental groups were designed in triplicate by combining two diets (control or supplemented with 0.2 % RELAQUAX) and two initial stocking densities: 5 and 25 kg/m$^3$, defined as low density (LD) and high density (HD), respectively. The trial was conducted over three months using *D. labrax* (n = 720; $w_i$ = 29.6 ± 5.9 g) housed in 500 L-tanks, where the density was adjusted by the number of fish and the effective volume of the tank. Fish were maintained under controlled temperature and photoperiod and fed to apparent satiation. Biometric assessments were carried out periodically to determine growth parameters and adjust feeding. At the end of the trial, samples were collected to analyse stress indicators, energy metabolism and hepatocytes' structure.

**Results and discussion**

Including RELAQUAX in the diet did not negatively impact fish growth. Regarding plasma metabolites (see Table 1), control group decreased significantly glucose, lactate and proteins levels under HD conditions. Dietary supplementation with the additive modulated this response, with plasma lactate and proteins levels remaining stable regardless of rearing conditions. The RELAQUAX group enhanced significantly plasma glucose values respect to control group under LD conditions. No significant differences were found in plasma triglyceride or cholesterol levels between the experimental groups. These results suggest that RELAQUAX supports metabolic stability and mitigates the negative effects of stocking stress by maintaining plasma lactate and proteins levels.

The control group increased significantly hepatic cholesterol under HD conditions ($p < 0.05$), with no other significant changes in liver metabolites (see Table 1). Conversely, RELAQUAX supplementation modulated hepatic response, increasing glucose values and decreasing hepatocyte area ($p < 0.05$), while other metabolites remained unchanged. Under LD conditions, RELAQUAX group enhanced significantly triglycerides and cholesterol compared to control group ($p < 0.05$). Under HD conditions, there was a significant increase in liver glucose and a decrease in hepatocyte area relative to control group ($p < 0.05$). These findings suggest that RELAQUAX supports liver metabolic flexibility by modulating glucose and lipid metabolism to maintain energy homeostasis. The decreased hepatocyte area may reflect an adaptive cellular response to stress modulated by the additive (Harper and Wolf, 2009).

**Conclusions**

Supplementing of aquafeed with RELAQUAX maintained plasma lactate and proteins levels under HD conditions, while increasing plasma glucose under LD. In the liver, promoted metabolic adjustments, with higher glucose and lipid levels depending on rearing density, and reduced hepatocyte area, suggesting histological adaptations to stress. Overall, RELAQUAX appears to support metabolic stability and liver flexibility, potentially enhancing physiological resilience under intensive farming conditions.

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**Table 1**. Plasma and liver parameters (mean ± standard error of mean). Different lowercase letters represent significant differences between stocking densities within the Control diet ($p < 0.05$). Different capital letters represent significant differences between stocking densities within the RELAQUAX diet ($p < 0.05$). Asterisks indicate significant differences between diets within the same stocking density ($p < 0.05$).

| | Low Density | | | High Density | | |
|---|---|---|---|---|---|---|
| | Control | RELAQUA X | | Control | RELAQUA X | |
| ***Plasma*** | | | | | | |
| Glucose ($mg \cdot dL^{-1}$) | 96.5 ± 3.9 a | 112.3 ± 5.3 A | * | 77.8 ± 4.1 b | 76.4 ± 3.5 B | |
| Lactate ($mg \cdot dL^{-1}$) | 28.9 ± 1.5 a | 30.1 ± 1.2 | | 25.5 ± 0.9 b | 27.9 ± 1.0 | |
| Triglycerides ($mg \cdot dL^{-1}$) | 846.3 ± 66.2 | 756.0 ± 57.3 | | 666.8 ± 50.0 | 693.3 ± 67.8 | |
| Cholesterol ($mg \cdot dL^{-1}$) | 250.7 ± 12.0 | 236.5 ± 12.8 | | 235.5 ± 13.2 | 240.1 ± 16.5 | |
| Proteins ($mg \cdot mL^{-1}$) | 52.2 ± 1.5 a | 49.6 ± 1.4 | | 47.5 ± 1.5 b | 47.9 ± 1.4 | |
| ***Liver*** | | | | | | |
| Glucose ($mg \cdot g\ fw^{-1}$) | 0.70 ± 0.07 | 0.60 ± 0.09 B | | 0.64 ± 0.08 | 0.89 ± 0.07 A | * |
| Glycogen ($mg \cdot g\ fw^{-1}$) | 16.3 ± 0.3 | 16.9 ± 0.5 | | 16.4 ± 0.4 | 16.7 ± 0.5 | |
| Triglycerides ($mg \cdot g\ fw^{-1}$) | 33.6 ± 1.9 | 49.8 ± 2.5 | * | 46.1 ± 5.2 | 48.9 ± 3.4 | |
| Cholesterol ($mg \cdot g\ fw^{-1}$) | 2.1 ± 0.2 b | 4.4 ± 0.5 | * | 3.9 ± 0.4 a | 3.6 ± 0.4 | |
| Hepatocyte area ($\mu m^2$) | 174.7 ± 2.6 | 178.6 ± 2.9 A | | 183.8 ± 2.9 | 162.5 ± 2.5 B | * |

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# PHYSIOLOGICAL RESPONSE OF THE GOLDEN GREY MULLET (*Liza aurata*) TO THE INCLUSION OF AN ENZYMATICALLY HYDROLYSED AND FERMENTED ALGAL BIOMASS

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## Introduction

The growing need to reduce the use of fishmeal and fish oil in aquafeeds has driven interest in sustainable alternatives such as algae, which are rich in essential nutrients and bioactive compounds. However, their high fibre content and rigid cell walls limit digestibility in fish. In order to solve this problem, biotechnological treatments like enzymatic hydrolysis and microbial fermentation help break down structural barriers and enhance nutrient availability (Hua et al., 2019). The golden grey mullet (*Liza aurata*), a euryhaline and omnivorous fish species well adapted to farming, is a promising candidate for aquaculture diversification (Crosetti and Blaber, 2016). This species requires a lower amount of dietary protein during the growth phase, enabling the employ of alternative ingredients and the reduction of production costs. For these reasons, this study assesses the physiological response of *Liza aurata* to bioprocessed algal biomass under feeding and fasting conditions.

## Materials and methods

The algal blend used in this study consisted of 30 % *Arthrospira* sp., 20 % *Chlorella* sp., 10 % *Microchloropsis* sp., 10 % *Tisochrysis* sp., and 30 % *Ulva* sp., provided by LifeBioencapsulation S.L. (Almería, Spain) and stored at -20 ºC until use. The biomass was processed through enzymatic hydrolysis (5h; carbohydrates, cellulase, and proteases), followed by microbial fermentation with *Saccharomyces cerevisiae* and *Bacillus subtilis* ($10^9$ CFU/mL each; 36 h).

A 62-day feeding trial was conducted at the Servicios Centrales de Investigación en Cultivos Marinos (SCI-CM, CASEM, University of Cadiz, Puerto Real, Cádiz, Spain; Facilities for Breeding, Supplying and Users of Experimental Animals; Spanish Operational Code REGA ES11028000312), testing, in triplicate, two experimental diets ($L_{50}$ and $L_{100}$), where 50 % and 100 % of fishmeal and fish oil were replaced by the processed algal product, and a control diet (CT) containing 15 % fishmeal and 5 % fish oil without algal biomass. At the end of the trial, fish growth was assessed. Additionally, the same fish were subjected to a fasting challenge during 9 days to evaluate their metabolic response to nutritional stress. Plasma and liver samples were collected both after the feeding period and following the fasting challenge to analyse metabolic indicators related to energy homeostasis.

## Results and discussion

The partial ($L_{50}$) and total ($L_{100}$) replacement of fishmeal and fish oil with enzymatically pretreated algae biomass had no negative impact on growth, indicating good overall acceptance and utilisation of the diets by the specimens of *L. aurata*. Despite the absence of significant differences in plasma metabolites, there was a trend towards lower plasma glucose, lactate and protein values in the experimental groups. This could indicate lower metabolic stress or improved energy efficiency associated with the use of the algal preparation.

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Although no significant changes were detected in liver tissue, a trend towards lower glucose concentration and higher glycogen storage in the $L_{50}$ and $L_{100}$ groups was observed, suggesting a more stable energy adaptation in specimens receiving these aquafeeds. Additionally, the $L_{100}$ dietary treatment resulted in a decrease in hepatic triglycerides, potentially reflected an increased use of lipids as an energy source or reduced lipogenesis. Notably, the hepatosomatic index decreased significantly ($p < 0.05$) in the $L_{50}$ group, despite a tendency to enhance hepatic triglyceride content. This apparent contradiction could be explained by a redistribution of hepatic triglyceride content in the $L_{50}$ group.

After the fasting period, no significant differences in biometric parameters were observed between treatments, indicating that the inclusion of the algae preparation did not negatively affect overall body condition following dietary restriction. However, groups that had been pre-fed experimental diets showed a significant reduction in plasma cholesterol ($p < 0.05$). As no differences in this metabolite were recorded during the feeding phase, this decrease suggests a differential metabolic response to fasting with an increased mobilisation of cholesterol (due to the previous dietary treatment). In terms of hepatic metabolites, while no statistically significant differences were detected, the $L_{50}$ group showed a trend towards greater triglyceride and glycogen accumulation, alongside lower glucose concentrations. This response suggests an optimisation of energy reserves, reflecting better metabolic resilience to fasting conditions.

**Conclusions**

Replacing fishmeal and fish oil with enzymatically hydrolysed and fermented algal biomass, either partially or entirely, appears to promote a favourable metabolic profile in *L. aurata* under both fed and fasted conditions. In particular, at intermediate levels of substitution ($L_{50}$), fish enhanced energy storage capacity and presented a more regulated metabolic response during periods of feed deprivation; suggesting an improved physiological resilience without compromising growth or condition.

**Acknowledgments**

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# REDUCING MICROPLASTIC RELEASE DURING CLEANING OF AQUACULTURE NETS

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Aquaculture uses large amounts of plastic for infrastructure and equipment, which likely contributes to microplastic (MP; <5 mm) release to the environment. In addition to feed pipes and cage collars, pen nets are a potential source for MP release. *In-situ* net cleaning, typically conducted using dedicated pressure washers, may exacerbate abrasion of nets and their coatings, leading to higher MP emissions during cleaning periods. As a result, fibres, as well as synthetic particles from net coatings, may be dispersed into the surrounding seawater.

A laboratory experiment simulating abrasion damage from net cleaning was performed to quantify and compare the relative amounts of MP released from common nylon nets and alternative net materials (HDPE, Dyneema, Entex), applying both new and used net samples. In addition, net samples with two different coatings, used to extend the material lifetime, were included. The abrasion tests were conducted using abrasion equipment from Buraschi, where the nets were subjected to a rotating abrasive drum in a water tank. Particles released into the water were washed and filtered (≥10 µm) before being quantified and physiochemically characterised using a combination of gravimetric measurements, microscope imaging and chemical fingerprinting by pyrolysis GC-MS.

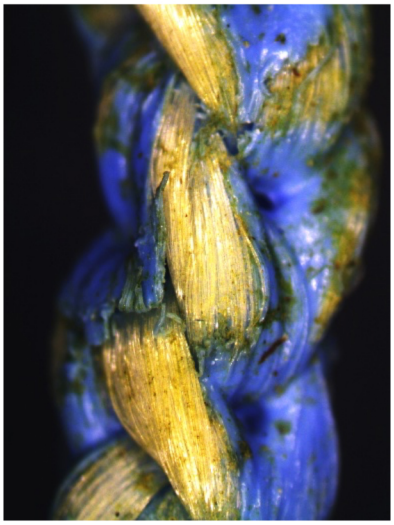

*Figure 1: Nylon net with coating damage after net cleaning*

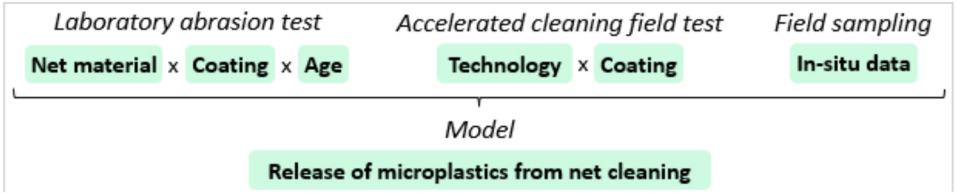


*Figure 2: Overview of the conducted experiments and collected data.*

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Results indicated higher MP release from the nylon nets compared to the HDPE, Dyneema and Entex nets. Coatings interacted differently with the various net materials, where there was no change in MP release from the coated Dyneema nets, but an increased particle release from the coated nylon nets. Used materials released more MP than new ones.

Based on these results, used nylon nets and new nylon nets with coating were chosen for an accelerated net cleaning test, conducted in full scale at sea. Here, we simulated 10 months of *in-situ* cleaning. Two novel cleaning technologies, cavitation-based cleaning and AUV based brushing, were compared to standard high-pressure based cleaning. The release of MP into the surrounding seawater, as well as abrasion of the coating from nets (Figure 1), were assessed. The MP release was determined using a combination of onsite filtration of seawater during the cleaning processes, and subsequent analysis of the retained particles by μFTIR spectroscopy to determine particle number, size distribution and polymer type.

To validate the results, we also sampled MP from seawater during a set of regular net cleaning events conducted with high-pressure washers at a commercial salmon farm site. Finally, we have modelled release from net cleaning based on the collected data (Figure 2) for different combinations of net materials, coatings, life stages and cleaning technologies to give the aquaculture industry a first indication of which material, coating and cleaning technology combinations may minimise current MP releases to the marine environment. These findings underscore the potential of material selection and maintenance practices in reducing the environmental impact of aquaculture operations.

**Acknowledgement**
The work is part of the project “Surveying microplastic release from aquaculture nets and ropes using different technologies for emission reduction (SMARTER)”, funded by the Norwegian Seafood Research Fund (FHF).

# EVALUATION OF THE IMPACT OF OXYGENATION SYSTEMS (OXYMAR®) ON HEALTH AND PRODUCTIVE PERFORMANCE IN SALMON FARMING SITES IN CHILE

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This study evaluates the impact of OXYMAR® oxygenation systems on the health and productive performance of Atlantic salmon farmed at open-sea sites in Chile between 2021 and 2024. A total of 620 farming centers, operated by 14 companies, were analyzed—comparing those using OXYMAR® technology with those that did not, including a subset affected by mortality associated with hypoxic events.

The primary objective was to determine whether the implementation of OXYMAR® systems improves sanitary and productive outcomes under low-oxygen conditions, thereby contributing to more sustainable aquaculture operations.

The results focus on the performance of sea sites using OXYMAR® in 2024, with geographic distribution across Los Lagos, Aysén, and Magallanes. The use of the technology was associated with a significant reduction in mortality rates (-24%), particularly at sites with frequent monthly oxygen supplementation.

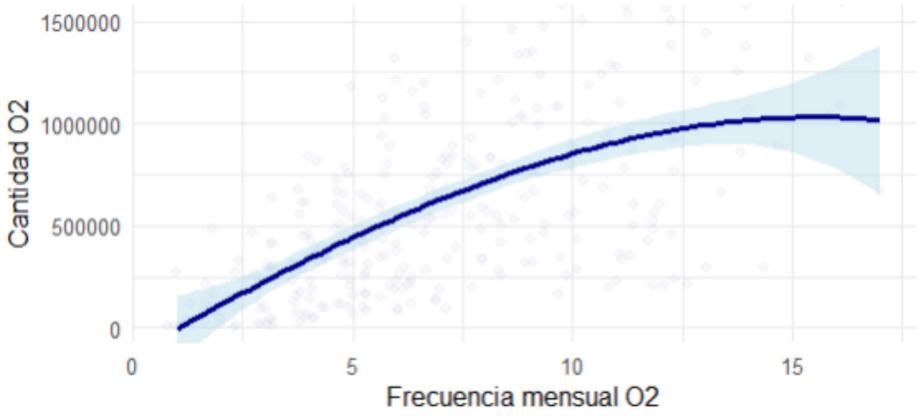


*Figura 6. Gráfico muestra una relación positiva entre la frecuencia mensual y consumo de O2*

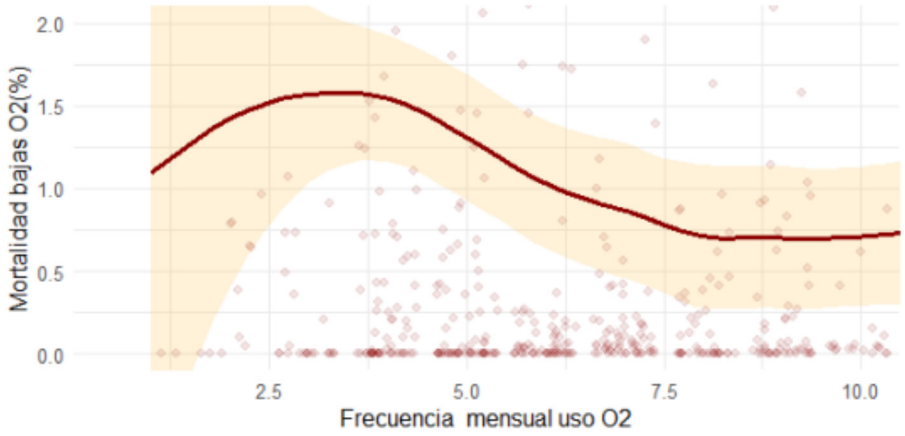


*Figura 7. La mayor frecuencia mensual de consumo se asocia a una menor mortalidad por bajas de oxígeno*

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Furthermore, sites using OXYMAR® demonstrated improved health indicators, including lower overall mortality and a reduced incidence of infectious diseases such as SRS and BKD. From a production perspective, these sites showed better feed conversion ratios (-2%), shorter production cycles (-5%), and higher specific growth rates (+4%), indicating greater operational efficiency.

These findings underscore the critical role of advanced oxygenation technologies like OXYMAR® in mitigating the effects of hypoxia, enhancing fish welfare, and supporting production stability in increasingly challenging marine environments. The evidence supports the strategic value of integrating oxygenation systems to optimize biological performance, reduce operational risks, and align aquaculture practices with global sustainability goals.

# BROAD-SPECTRUM ANTIVIRAL POTENTIAL OF MARINE INVERTEBRATE EXTRACTS: A BLUE ECONOMY APPROACH

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**Introduction**

Marine ecosystems are a rich source of bioactive compounds, many of which remain largely unexplored. Among marine organisms, invertebrates are particularly notable for their capacity to produce unique secondary metabolites with potential applications (Ruiz-Torres et al. 2019). Viral infections are one of the major challenges to both human health and aquaculture. In aquaculture, viral pathogens cause significant economic losses and threaten the sustainability of fish farming systems, while in human health, effective antiviral treatments remain limited for many viral diseases. This highlights the importance of discovering new antiviral compounds, especially those with broad-spectrum activity and versatility across different sectors (Riccio et al. 2020).

This study investigates the antiviral potential of extracts from four marine invertebrates that target viruses relevant to humans and fish. By exploring the therapeutic potential of marine biodiversity, this research supports the European Blue Economy approach, which is based on sustainably using ocean resources to drive innovation in health and economic development.

**Materials and Methods**

Extracts were prepared from four marine invertebrate species: two nudibranchs (NA and NB), one holothurian (PS) and one soft coral (CR). Organic solvents were used for extraction, and the resulting dry extracts were solubilized in dimethyl sulfoxide. Eight viruses were selected for *in vitro* antiviral screening:

- Human respiratory viruses: human coronavirus (HCoV-229E), human metapneumovirus (hMPV), respiratory syncytial virus (RSV), and influenza A virus (IAV).
- Fish viruses: viral hemorrhagic septicemia virus (VHSV), cyprinid herpesvirus 3 (CyHV-3), tilapia lake virus (TiLV), and common carp paramyxovirus virus (CCPV).

Each viral strain was incubated at virus-specific multiplicities of infection (MOIs) for two hours at the optimal infection temperature. Then, to evaluate antiviral activity, three types of assays were performed: in some cases, viral particles were pre-incubated with the extracts before being added to susceptible cells; in others, cells were pre-treated with the extracts prior to infection. In additional assays, cells were treated throughout the entire infection cycle. Antiviral activity was evaluated by quantifying the percentage of infection inhibition compared to untreated controls for each virus–extract combination.

**Results**

Extracts from NB and NA showed the highest antiviral activity, significantly reducing infection and replication across a broad range of human and fish viruses. These extracts consistently showed greater infection inhibition compared to the other two extracts indicating higher efficacy. Although PS exhibited antiviral activity against certain viruses, it also showed cytotoxic effects at some concentrations, which may limit its applicability.

**Discussion**

The strong antiviral effects of NB and NA extracts suggest the presence of novel and potent bioactive compounds in these two species. These findings are particularly promising for aquaculture, where effective antiviral agents are critically needed. The observed efficacy against human viruses further highlights their potential application in therapeutical development.

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The design of the trials enables us to formulate very tentative hypotheses about the mechanisms of action. The antiviral effect observed after virus extracts pre-incubation suggests direct virucidal activity, possibly through inactivation of viral particles or interference with their ability to attach to host cells. The pre-treatment of cells suggests a protective cellular effect, such as modulation of viral receptors or activation of cellular defense pathways (Aoki-Utsubo et al. 2018).

Together, these results suggest that NB and NA are promising candidates for the development of broad-spectrum antiviral agents. Further research is, however, required, with the next steps of the investigation focusing on isolating and characterising the active compounds, as well as elucidating their chemical structures and specific molecular targets.

**Conclusion**

This study identifies two marine invertebrates, NB and NA, as promising sources of broad-spectrum antiviral agents. Their demonstrated efficacy against both human and fish viruses highlight their potential for cross-sector therapeutic development, spanning human medicine and aquaculture. Approaches aligned with the Blue Economy, such as the exploration of marine bioactive compounds, will be essential to advancing these findings toward practical, real-world applications. Continued research focused on the isolation, characterization, and mechanism of action of the active compounds will be key to unlocking their full potential.

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# COASTAL SPRING WATER ON THE VALENCIAN COMMUNITY COAST (E SPAIN)

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**Introduction**

Coastal aquifers of the Valencian Community are currently heavily overexploited. Some freshwater springs, such as Peñiscola, are still recognized, indicating the existence of others that currently do not discharge into the sea but into a coastal freshwater lagoon, such as the Albufera de Valencia, or others that are only active during rainy periods, such as Toix (Tent-Manclús et al., in press).

**Discussion**

In the 17th century, Gaspar Escolano (1611) made a description of the coasts of the Kingdom of Valencia, described different sources of fresh water along the coast. In Peñiscola (Province of Castellon), a water spring near where the waves break became salinized and then returned to be fresh water, being one of the first documented mentions of natural coastal salinization and its reversal. The Albufera of Valencia was a lagoon of sea water connected to the sea that had freshwater springs, used by fishermen. Today, some springs called "ullals" are still active and already pour into a coastal lagoon of fresh water instead. The change was produced by the human activity at the end of the 18th century (Sanchis Ibor, 2010).

Finally, in Alicante, he mentions the Agua Dulce cove in Denia and the sandbank of Rio Seco. Special mention has to the description of the important coastal spring of the Morro de Toix seacliff, now mainly inactive most of the year around and only active after major rain episodes as the one on March 26th, 2025 were the under sea-level springs worked as can see in the Figure 1. The Escolano's description of Morro de Toix mentioned that the big current produced by the spring did not allow the small boats to get closer to it. Also, Cavanilles (1797) describe as active coastal spring.

**Conclusion**

The ancient coastal spring water of the Valencian Community of 400 years ago from historical sources had been revised. Most of the springs are not discharging due to the great anthropic modification of the 20th-21st centuries.

**Acknowledgments**

This work has been funded by NextGenerationEU (PRTR-C17.I1) of the European Union, proyect of the Generalitat Valenciana GVA-THINKINAZUL/2021/039, and the Alicante University Group VIGROB21-053.

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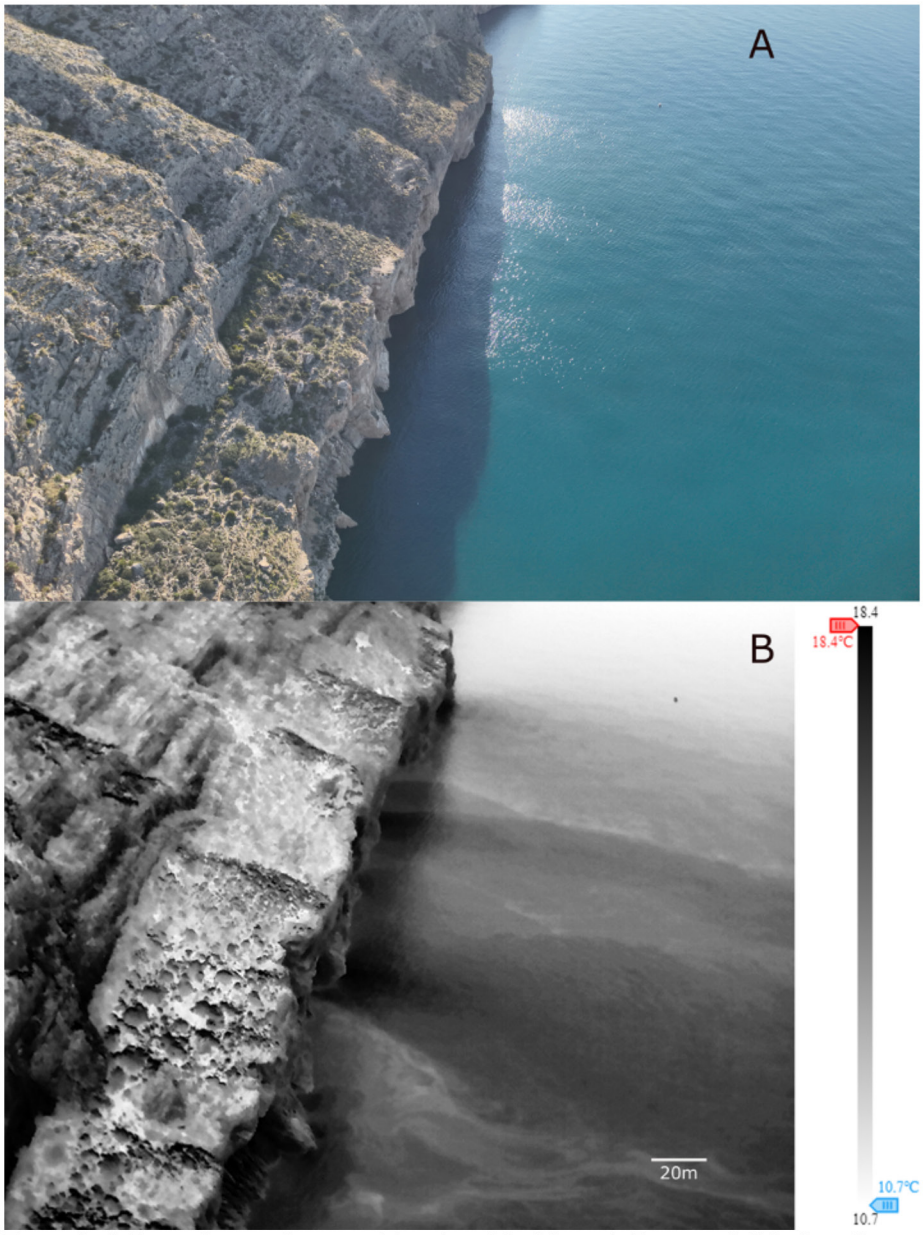


*Figure 1. A) True color aerial image of the edge of the Morro de Toix sea-cliff. In the reflections of the sun, it is appreciable a current that rises sharply from the outcrop. B) Same image as A) in the thermal camera (hot black) in C°. The plumes are reflected in the current that can be noticed in A image.*

# DEVELOPMENT OF A USV PLATFORM TO STUDY COASTAL SPRING

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**Introduction**

One of the major limitations of traditional bathymetric and Side Scan Surveys are the shallow waters, less than 5 m depth, where the campaign ships have poor maneuverability, and the waves are increased. Unmanned or Uncrewed Surface Vessels (USV) are remote controlled vehicles or autonomous vehicles that can work in shallow depth areas, narrow channels, and hazardous areas. They are the best option for shallow waters if the marine weather conditions are appropriate, as low waves and almost non-tide currents.

Our group of the University of Alicante has developed a low-cost USV platform to be used in coastal, rivers and lake studies (Ronda et al, 2023; Benabdeloued et al., 2025). The main advantage is an affordable and easily adaptable USV, not dependent on external developers, and changeable on-demand.

**USV description**

The USV is based on an Arduino mainboard to control the Electronic Speed Control (ESC) of the thrusters, the GPS and the antennas for sending signals and two video-cameras, one underwater, facing down, and other above the water, facing to the front. The ship was designed to be easily transported and operated in areas with limited access, such as densely vegetated lake margins, as the thruster are protected by a steel net. It can be equipped with various sensors, during different development phases a Furuno single-beam was used, and at the moment is updated with a Lowrance Elite FS 3-in-1 single-beam, down-scan and side-scan sonar with an own GPS plotter (Benabdeloued et al., 2024).

To test the platform, it was deployed in different study sites: as spring ponds (Ronda et al., 2023), and the sea-cliff (Bonomo et al., 2025). For this last one, the side-scan sonar equipped USV provided detailed seabed sonar images and lateral underwater vertical sea-cliff, uncovering the underwater excavation of the cliff and the nearby seabed with karstic features such as caves and blocky bottom. The system shows the effectiveness in collecting high-resolution bathymetric data, even in challenging environments.

**Discussion and conclusion**

The key advantage of the system lies in its low cost, adaptability, and the ability to collect data in areas that would otherwise require expensive equipment. This research highlights the potential of USVs for advancing aquatic and coastal studies, offering a scalable solution for both academic and applied environmental research. Our system is in a continuous development, as in the near future will be updated with new sonar transducers.

**Acknowledgments**

This work has been funded by NextGenerationEU (PRTR-C17.I1) of the European Union, proyect of the Generalitat Valenciana GVA-THINKINAZUL/2021/039, and the Alicante University Group VIGROB21-053.

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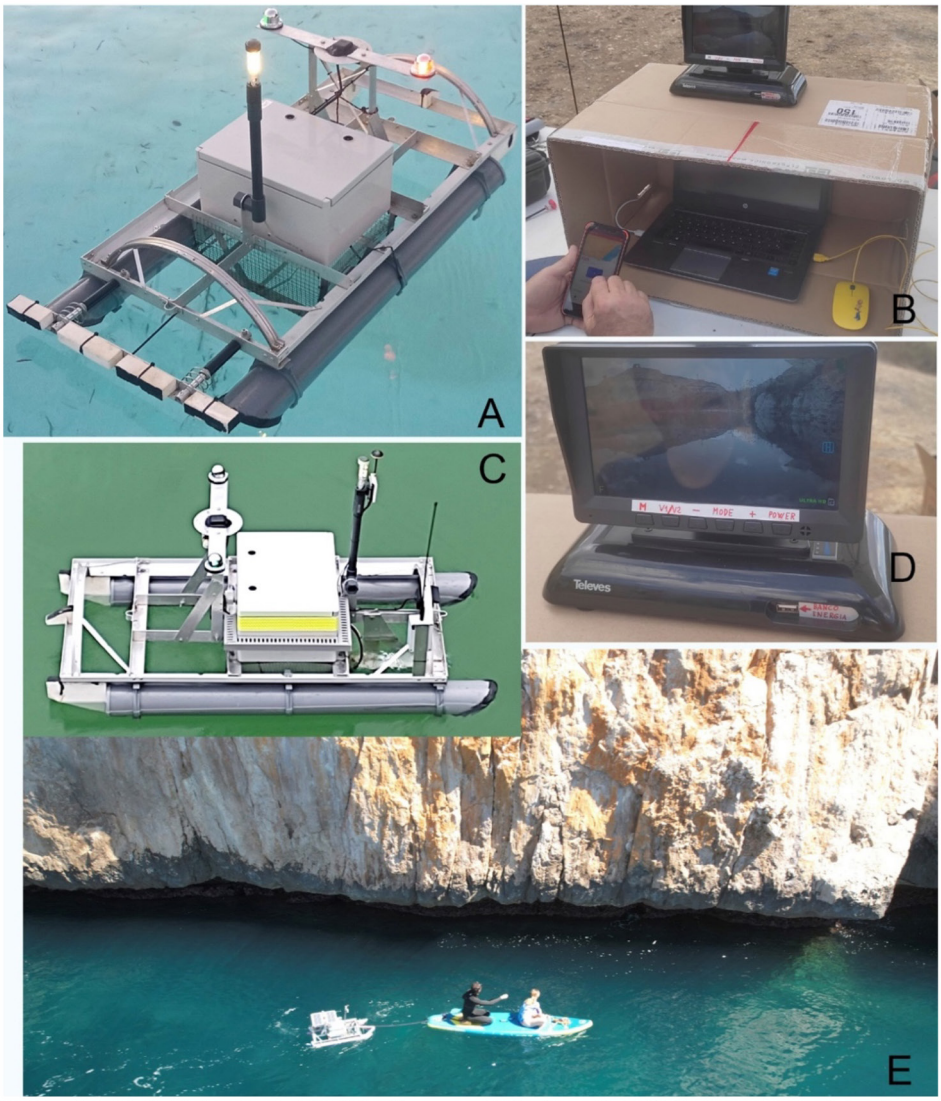


Figure 1. A) Self-developed USV (Unmanned Surface Vehicle) in development phase I, with position, starboard and port side lights and GPS tracker. B) Custom control working station. C) USV development phase II, the platform has been improved with a submerged and a frontal camera (to the right) plus two antennas, one for amplify the RC communication signal and the other for real-time video broadcast. D) Real-time video display of one-at-time cameras installed on USV. E) USV man-powered example in a Side Scan Scan survey of a sea-cliff.

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# CLONING AND CHARACTERIZATION OF A *VASA* HOMOLOG IN THE GIANT GROUPER (*Epinephelus lanceolatus*) AND ITS SPECIFICALLY EXPRESSION IN GONAD

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The *vasa* gene has been the most commonly used gene marker for germ cells in vertebrates, particularly in fish. This study aimed to clone and characterize a *vasa* homolog in the giant grouper (*Epinephelus lanceolatus*), which was designated as *Elvasa*. Our results showed that *Elvasa* contained predicted regions sharing consensus motifs with the *vasa* family in teleosts, including arginine-glycine (RG) repeats, arginine-glycine-glycine (RGG) repeats, ATPase motifs, and a DEAD box. Phylogenetic tree analysis showed that *Elvasa* was located on a branch of the *vasa* family tree and was clearly distinct from PL-10. Reverse transcription PCR was performed to determine the tissue distribution of *Elvasa*. Our results showed that, using cDNA preparations from the heart, liver, spleen, intestine, kidney, muscle, and gonads (ovary and intersex), *Elvasa* transcripts were detected only in the gonads. Histological examination and *in situ* hybridization were performed on the ovary, and the results showed strong positive signals for *Elvasa* in primary oocytes. Therefore, *Elvasa* showed specifically expressed in gonad, which could be used for germ cell marker. Therefore, Elvasa was specifically expressed in the gonads and could be used as a germ cell marker.

# INNOVATIVE SUSTAINABLE AQUACULTURE PRACTICES: SALMON AND MICROALGAE LAND-BASED IMTA

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## Introduction

The Farm-to-Fork Strategy of the European Green Deal acknowledges the potential of algae to become an important source of alternative low-carbon footprint protein and contribute to improving the sustainability and competitiveness of the aquaculture sector (Gallego et al., 2025). Within this context, we have developed and demonstrated the integration of fish and algae cultivation in coupled, land-based RAS/IMTA (Recirculating Aquaculture Systems/ Integrated MultiTrophic Aquaculture) systems. This concept aims to minimize energy and nutrient losses and maximise resource efficiency by closing the nutrient loop.

## Material and Methods

In the land-based RAS/IMTA for salmon production connected to microalgae cultivation, we studied the nutrient uptake and microalgae biomass productivity on the RAS effluent water. The nitrogen and phosphorous concentrations of the RAS effluent water change over time and are highly dependent on the operation conditions of the production systems. Moreover, the nutrient concentrations are generally lower than the concentrations used for fertilization at industrial scale microalgae production. Therefore, we explored the possibility of microalgae cultivation systems adaptable to different hydraulic retention times for the water and the algae, i.e. a biofilm reactor and a perfusion system. Perfusion cultivation was tested through the integration of a membrane filtration system. The performance was compared to continuous cultivation with and without additional fertilization. The microalgae species *Chlorella vulgaris* and *Phaeodactylum tricornutum* were used for the freshwater experiments and brackish/seawater experiments respectively.

Furthermore, a life cycle assessment was conducted to evaluate the potential environmental impacts for the different microalgae cultivation methods. A preliminary economic evaluation used to determine the differences in capital expenses and operational expenses between the two microalgae cultivation modes.

## Results and Discussion

We successfully demonstrated nutrient remediation for RAS effluent water, during the freshwater, brackish water and seawater stages of the salmon production, for both microalgae species *Chlorella* and *Phaeodactylum*. Results showed that high microalgae productivities could be achieved (up to 2.15 g $L^{1}$ $d^{-1}$), as well as high nutrient uptake rates (up to 1888 mg $NO_3$ $L^{-1}$ $d^{-1}$ and up to 252 mg $PO_4$ $L^{-1}$ $d^{-1}$). A steady state microalgae cultivation with stable optical densities and dry weights cultivation was obtained for all experiments. The results from the lab experiments were used to update and expand an existing model for 1 ha microalgae cultivation (Böpple et al., 2025; Vázquez-Romero et al., 2022). A life cycle assessment (LCA) and preliminary economic assessment of the upscaled model showed that perfusion cultivation appears to have a lower environmental impact for treating RAS effluent water with low nutrient concentrations, but leads to increased operational (6 %) and capital expenses (up to 60 %) due to additional equipment and energy demands. The infrastructure of the photobioreactor and the electricity needed during cultivation contribute the most to the environmental impacts.

Although the potential application of microalgae in aquaculture feed could improve the sustainability of fish feed (Ansari et al., 2021), a clearer regulatory framework is needed regarding microalgae cultivated on ‘waste streams’ from aquaculture for food and feed production.

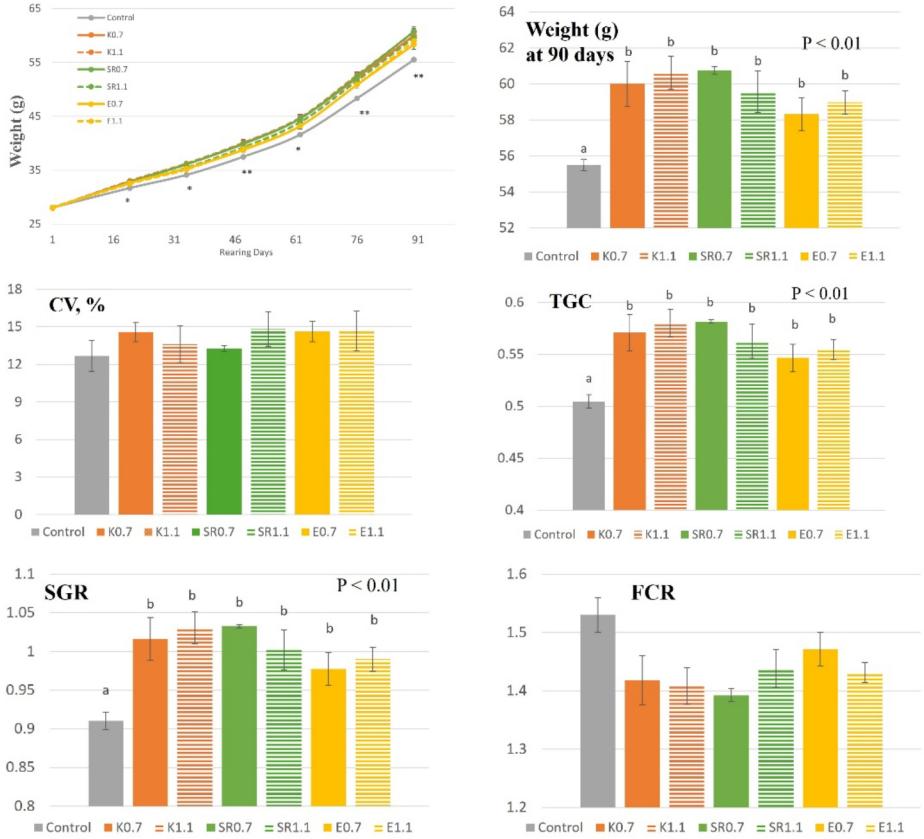


Figure 1: Growth performance of gilthead seabream after 90 days of rearing.

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# PARTIAL REPLACEMENT OF FISH MEAL WITH HYDROLYSED FEATHER MEAL IN THE DIET OF GIANT RIVER PRAWN (*Macrobrachium rosenbergii*) DURING THE NURSERY PHASE

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## Introduction

Freshwater species like the giant river prawn (*Macrobrachium rosenbergii*) are a promising alternative to other farmed crustaceans due to their high market value and significant production, which reached 294,000 tons in 2020 (2.6% of total crustacean farming) (FAO, 2022). However, optimizing their nutrition remains a challenge, particularly in identifying sustainable protein sources.

Among the alternatives to fish meal, hydrolysed proteins from processed animal by-products (PAPs) such as hydrolysed feather meal (HFM) could offer a viable solution, improving feed efficiency while reducing waste. Despite their potential, research on the use of PAPs in freshwater prawn diets, particularly during early developmental stages like post larvae, is still limited.

This study evaluates the effects of HFM inclusion in *M. rosenbergii* post larvae diets, assessing growth performance, digestive enzyme activity, antioxidant status, and whole-body composition.

## Material and Methods

A total of 1600 *M. rosenbergii* postlarvae (0.021 ± 0.006 g) were distributed into 20 aerated 250-L tanks, each connected to a recirculating system with a Bead filter (1200 L $h^{-1}$ flow rate). Prawns were randomly assigned to five diets with different HFM inclusion levels (0.0%, 1.5%, 3.0%, 4.5%, 6.0%) and four replicates per diet. Diets were formulated to be isonitrogenous (44.2 ± 0.4% crude protein) and isolipidic (14.8 ± 0.1% crude lipids). Ingredients were ground (0.5 mm sieve), mixed, pelleted, and feeds stored at 4 °C until use. A 32-day feeding trial was run, which included five daily feedings (9:00, 11:00, 14:00, 17:00, 21:00). Tanks were siphoned before the first feeding to remove faeces and uneaten food. The initial feeding rate (10% of biomass) was daily adjusted daily based on consumption. Mortality was recorded twice daily. A 12 h light/12 h dark photoperiod was maintained. Temperature and dissolved oxygen were measured daily; total ammonia nitrogen and nitrite, three times a week; hardness and alkalinity, biweekly. Water parameters were within the recommended range for *M. rosenbergii* farming (mean ± SD): temperature 29.35 ± 0.91 °C, dissolved oxygen 6.10 ± 1.05 mg $L^{-1}$, pH 8.34 ± 0.09, hardness 108 ± 19 mg $CaCO_3$ $L^{-1}$, alkalinity 107 ± 11 mg $CaCO_3$ $L^{-1}$, total ammonia nitrogen 0.011 ± 0.015 mg $L^{-1}$, and nitrite 0.003 ± 0.29 mg $L^{-1}$. At the end of the trial, survival, final weight, length, antenna length, biomass gain, and feed conversion rate were assessed. Hepatopancreases from 100 prawns (20 per diet) were collected to analyse digestive enzyme activity, antioxidant activity, and lipid peroxidation. Data were tested for homogeneity (Levene's test) and normality (Shapiro–Wilk's test). One-way ANOVA followed by Tukey's test was used for analysis. Results are expressed as mean ± SEM, with $p<0.05$ considered statistically significant.

## Results and discussion

Survival rates ranged from 73.3±5.4 % to 83.3±3.8 % without significant differences among treatments ($p>0.05$). All treatments with HFM inclusion showed significant higher final weight in comparison with the control treatment ($p<0.05$). Final weight, final length, and biomass gain were significantly higher in prawns fed diets with 6.0% HFM ($p<0.05$) and the feed conversion rate was significantly improved by the same diet ($p<0.05$). Additionally, prawns fed diets containing 4.5% or 6.0% HFM inclusion had significantly longer antennae ($p<0.05$), suggesting improved welfare conditions (Furtado et al., 2015).

Antioxidant status did not change according to the dietary treatment ($p>0.05$), suggesting that HFM did not induce oxidative stress or disrupt prawn redox balance. In details, thiobarbituric acid reactive substances (TBARS) averaged 0.94 ± 0.13 µM/mg protein; reduced glutathione (GSH) 1.70 ± 0.37 µM/mg protein; glutathione S-transferase (GST) 0.18 ± 0.04 nMol tioeter/min/mg protein; glutathione peroxidase (GPx) 0.88 ± 0.16 µM NADPH/min/ mg protein; and glutathione reductase (GR) 0.73 ± 0.18 µM NADPH/min/mg protein.

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The lack of differences in digestive enzyme activity according to the diet suggests that HFM inclusion of up to 6% did not significantly alter the digestive physiology of prawns post larvae, both at carbohydrate (maltase: 11341 ± 2902 U/mg protein, on average; sucrase: 4266 ± 1233 U/mg protein) and protein (chymotrypsin: 4.94 ± 0.73 µmol/min/mg protein; trypsin: 1.54 ± 0.25 nmol/min/mg protein) digestion level. On the other hand, in Pacific white shrimp (*Penaeus vannamei*) fed 6% of HMF during the grow-out phase, higher levels of trypsin activity were recorded compared with the control diet, suggesting a potentially enhanced protein digestion (Negrini et al., 2024a), while no changes were observed in Pacific white shrimp fed similar diets during the nursery phase (Negrini et al., 2024b).

**Conclusions**

Hydrolysed feather meal inclusion up to 6% improved growth performance, feed conversion, and welfare in giant freshwater prawns without compromising digestive enzyme activity or antioxidant status. These findings suggest that HFM is a viable alternative protein source that supports efficient digestion and shrimp health. Additionally, its use promotes circularity also in crustacean aquaculture by valorising poultry by-products, reducing reliance on traditional protein sources, and enhancing the sustainability of prawn production.

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# NUTRITIONAL INTERACTIONS BETWEEN ZINC AND OMEGA-3 FATTY ACIDS: IMPACTS ON SKIN REPAIR AND SKELETAL DEVELOPMENT IN ATLANTIC SALMON

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**Introduction**
The growth of the salmonid aquaculture industry has driven major changes in feed composition, leading to diets with higher lipid content and increased use of plant-based ingredients. As a result, these diets often contain reduced levels of the omega-3 fatty acids EPA and DHA. Additionally, due to environmental concerns and regulatory restrictions, zinc (Zn) content in salmon diets has also been reduced. The aim of this study was to investigate the interactions between dietary Zn and omega-3 fatty acids in relation to growth, body composition, skin health, and bone development in Atlantic salmon, using a combination of *in vivo* and *in vitro* approaches.

**Material and Methods**
Atlantic salmon parr (initial weight: 5 g) were fed six experimental diets for five weeks in triplicate tanks. These diets varied in Zn (120, 180, and 220 mg/kg, referred to as low [LZn], medium [MZn], and high [HZn], respectively) and EPA+DHA content (1% or 3%, referred to as low [LΩ] or high [HΩ], respectively). At the end of the feeding trial, skin and vertebra samples were collected from 10 fish per tank, flash-frozen in liquid nitrogen for chemical analyses, and fixed in 10% formalin for histological analysis. An additional 10 fish per tank were frozen at −20°C for whole-body fat analysis, fatty acid composition, mineral content, and vertebral X-ray and CT imaging.

To assess wound healing, 36 fish from each dietary group were transferred to a common tank and continued on their respective experimental diets for a 6-week wound healing trial. Two standardized skin wounds were inflicted: a superficial wound created by brushing five times with a toothbrush horizontally, and a deep wound involving a vertical incision approximately 2 mm long and 2 mm deep. Tissue samples from the wound areas were collected at 4 days and 6 weeks post-wounding for gene expression and histological analysis.

Three *in vitro* experiments were also conducted, using scales collected from six fish. Five scales per fish were placed in each well, totalling 30 scales per treatment, tested in triplicate. Four conditions were evaluated: control, 0.5 mg/L Zn, 10 µM DHA, and a combination of 0.5 mg/L Zn + 10 µM DHA. The effect on cell migration was measured as the ratio of scales with migrating keratocytes to those without.

**Results and discussion**
The experimental diets had no significant effects on growth performance, as measured by standard growth rate (SGR) and thermal growth coefficient (TGC). As expected, the dietary content of EPA+DHA was reflected in whole-body fatty acid composition, and Zn concentrations in the whole body, skin, and vertebrae increased proportionally with dietary Zn levels. Interestingly, a synergistic effect between Zn and EPA+DHA was observed, particularly at the highest inclusion levels (3% EPA+DHA and 220 mg/kg Zn), resulting in elevated tissue Zn concentrations. Fish receiving this diet also exhibited the highest bone density. Analysis of scale maturation in the skin showed that fish fed the lowest Zn levels (HΩ/LZn and LΩ/LZn) had the least mature scales, while those fed high Zn and high omega-3 (HΩ/HZn) had the most mature scales by the end of the trial. Spinal deformities were observed in 6% of the fish, with twice as many deformities occurring in the LZn groups compared to those fed MZn or HZn, regardless of EPA+DHA content.

Wound healing assessments of mechanically induced injuries (deep cut, superficial wound, and surrounding skin) showed faster scale regeneration in fish fed diets containing the highest levels of both Zn and omega-3. Additionally, the *in vitro* model using salmon scales demonstrated that Zn and DHA both enhanced the migratory capacity of keratocytes. Since rapid keratocyte migration and scale regeneration are critical for wound healing and maintaining skin integrity, these findings underscore the role of these nutrients in fish health.

**Conclusion**
Overall, this study highlights the importance of balancing dietary Zn and omega-3 fatty acids to support optimal growth, skin and bone development, and overall welfare in Atlantic salmon.

# INCREASED DIETARY PHOSPHOLIPISDS OF MARINE OR PLANT ORIGIN, BUT NOT OF ANIMAL ORIGIN, ENHANCE GROWTH PERFORMANCE OF JUVENILE GILTHEAD SEABREAM *Sparus aurata*

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## Introduction

It is already widely accepted that fish larvae need high levels of phospholipids (PLs) in their nutrition to ensure optimal growth. Phospholipids are important structural components of cellular membranes, and during the larval stage the formation of tissues and organs is still underway (Sargent et al. 2002; Saleh et al. 2012). On the other hand, there is no solid data regarding phospholipid needs of fish in the juvenile stage or later. This may be because there is some evidence, albeit limited, of the existence of a phospholipid biosynthesis mechanism in fish (Sargent et al. 2002). Currently in Mediterranean aquaculture genetic selection programs are in place that have achieved improved growth rates in fish (Ferosekhan et al. 2022). Knowing that faster weight gain also means faster tissue growth, questions are raised about whether the current nutritional design of feeds are adequately covering the needs of fish in structural nutrients, such as phospholipids. The aim of the present study is to investigate if the increased nutritional intake of PLs will affect production and physiological characteristics of gilthead seabream juveniles (*Sparus aurata*). Simultaneously, different PL sources will be explored (marine, plant, animal) regarding their efficiency.

## Materials and methods

Seven different diets (treatments) were formulated and produced, of which one was used as a Control diet (typical composition of commercial diets, PL 0.5%). The rest of the diets contained, in pairs, different phospholipid sources (krill-K, soy/rapeseed-SR, egg-E) in two levels (PL 0.7% and PL 1.1%). Going forward the diets will be referenced to as K0.7 and K1.1, SR0.7 and SR1.1 and E0.7 and E1.1. Twenty-one homogeneous groups of 20 juvenile gilthead seabream with initial body weight (BW) 28.0 ± 0.13 g (triplicated groups for each treatment) were used. Fish are fed a restricted ration according to water temperature and BW. Mean water temperature is 17.3 ± 0.7 $^{o}$C. When the fish have tripled their initial weight, the trial will end, and they will be weighed and sampled. The growth parameters that are being investigated include: fish weight (W) and its coefficient of variation ($CV_w$), specific growth rate (SGR), feed conversion rate (FCR) and thermal growth coefficient (TGC). For TGC, degree days (DDs) are also calculated (1538.25 for 90 days of rearing). Additionally, the physiological condition of the fish will be evaluated by analyzing blood samples (cholesterol, glucose, albumin, triglycerides, total protein). The trial is ongoing, therefore the results presented below concern the growth for the first 90 days of the experimental rearing.

## Results and Discussion

Fish weight was significantly lower in the Control diet after 90 days of rearing, regardless of the phospholipid inclusion level and origin (Figure 1). This was already observed only two weeks after the beginning of the trial. No differences were observed for $CV_w$ and FCR among diets. SGR and TGC for all experimental diets were equally significantly higher than Control diet (Figure 1). The results for the first 90 days of rearing suggest that gilthead seabream juveniles have higher phospholipid needs than previously reported. It is also encouraging that despite low water temperatures, there is still an obvious improvement in growth. Regarding the efficiency of the different origins of PLs, the present absence of differences does not permit a conclusive evaluation yet. However, it seems that the inclusion of egg PLs doesn't seem to be as efficient as the inclusion of marine and plant PLs. The limited literature data does not permit adequate comparisons with present results, especially when most studies focus on increasing the phospholipid levels of feeds that have a very low fish meal percentage. However, present results suggest that gilthead seabream juvenile growth is affected by the level of dietary phospholipids and indicate a differential effect of PL origin.

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# DEVELOPING AND TESTING A DIGITAL TWIN FOR A BRACKISHWATER AQUAPONIC FARM

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Aquaponics is an innovative food production technique, deriving from the integration of recirculating aquaculture system (RAS) and hydroponics, suitable for development of urban and peri-urban food productions[1,2]especially in Europe. Whilst aquaponics broadly combines recirculating fish culture with hydroponic plant production, the application of the term aquaponic is broad and many technologies claim use of the name. Combining fish culture with aquatic-based, terrestrial plant culture via aquaponics may be better defined via its nutrient resource sharing credentials. Aquaponics applies several principles including, but not limited to, efficient water use, efficient nutrient use, lowered or negated environmental impact and the application of biological and ecological approaches to agricultural fish and plant production. Water sources are important so that the nutrients required for fish and plant production are available and balanced, and system water chemistry is paramount to optimised fish and plant production. Systems may be configured in several ways, including those that are fully recirculating and those that are decoupled. Aquaponics importantly seeks to apply methods that provide technical, biological, chemical, environmental and economic advantages.",",container-title":"Aquaponics Food Production Systems",",event-place":"Cham",",ISBN":"978-3-030-15942-9",",language":"en",",note":"DOI: 10.1007/978-3-030-15943-6_5",",page":"113-143",",publisher":"Springer International Publishing",",publisher-place":"Cham",",source":"DOI.org (Crossref. However, high CAPEX/OPEX of aquaponics require a careful selection of highly remunerative products in order to achieve economic sustainability. Aquaponics practiced in brackishwater conditions seems to offer interesting options for sectorial diversification.

The present work, performed in the framework of the Interreg Italy-Slovenia project BEBLUE, aims at studying the functioning of a pilot aquaponic farm rearing gilthead seabream (*Sparus aurata*), in combination with macroalgae (*Ulva rigida*) and samphire (*Salicornia europea*). The approach was based on the precision fish farming framework[3], combining monitoring efforts with the development of a digital twin of the aquaponic farm. The digital twin is a dynamic mathematical model describing the evolution of the aquaponic system, through the simulation of: 1) the key metabolic processes of fish, primary producers and bacteria; 2) the water balance and the transport process of the suspended solids and the dissolved metabolites; 3) the chemical equilibria regulating solution acidity. The model includes four typical components of an aquaponics system, namely: 1) Fish Tank (FT); 2) Mechanical Filter (MF); Biofilter (BF) amd GreenHouse.

The model was tested by comparing its outputs with the experimental data gathered by monitoring the pilot system over a 4 months period, between April 15$^{th}$ and August 15$^{th}$ 2024. Model results (Figure 1) successfully reproduced the observed seabream and samphire individual weight and total biomass, as well as the concentration of the abiotica variables: Dissolved Oxygen and Total Ammonia Nitrogen are shown in Fig. 1. Simulated and observed nitrate concentration, not shown, presented in increasing trend, highlighting that the cultivated crop surface could assimilate only a small fraction of the N metabolized by the fish. This output can be of interest for system design, supporting a correct dimensioning. Observed fluctuations in oxygen concentration were reproduced satisfactorily, showing a slightly lower $O_2$ concentration in the biofilter, although compensated by an adequate reaeration rate. Also in this case, the model could be of help for dimensioning the air insufflation systems and producing early warning of oxygen drifts. The three variables defining the carbonate system, total alkalinity, DIC, and pH, are correctly represented, with a slight underestimation of pH during the initial phase of the simulation. The capability of simulating the carbonate system dynamics is useful for an informed optimization of pH conditions which are contextually adequate for the biofilter and the crops.

Modelling and monitoring results were discussed, to derive a set of recommendations for the development of aquaponics in brackish water.

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# OPERATIONAL OCEANOGRAPHY AND MSP DATA SUPPORTING AZA PLANNING: THE CASE STUDY OF EMILIA-ROMAGNA REGION

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Allocated Zones for Aquaculture (AZA) represent a strategic tool for the sustainable development of the sector in the Mediterranean Sea (Macias et al., 2019). AZA identification and planning, currently being carried out throughout the basin, are facing relevant challenges, such as coexistence with conservation priorities and existing maritime activities, as well as climate resilience. In this framework, the use of freely accessible geospatial data represents a strategic asset.

The present contribution focuses on a case study of AZA planning, reviewing how mathematical modelling and geostatistical analysis supported data processing and informed planning choices. The case study was carried out within the AZA-RER project (PN FEAMPA 2021-2027), considering the Emilia-Romagna region, the most important Italian region in terms of shellfish production (*Mytilus galloprovincialis, Ruditapes phylippinarum*, *Crassostrea gigas*). Data sources were Copernicus Marine Service (CMS) and European Marine Observation and Data NETwork (EMODNET), complemented by data on operational oceanography made available by the local Environment Protection Agency (ARPAE). Open data exploitation by means of different mathematical and statistical models were compared, carrying out an analysis of the spatial maps produced, and identifying the existing knowledge gaps. Future developments are discussed, in relation to the application of the proposed data exploitation framework for participatory mapping within the ongoing stakeholder engagement process.

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# FISH WELFARE ON THE LINE: WHY OPERATIONAL INDICATORS AND STUNNING METHODS MUST BE NEUROPHYSIOLOGICALLY VALIDATED

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## Introduction

Slaughter is a major welfare concern in aquaculture, with an estimated 78–171 billion fish killed annually. Humane slaughter methods must ensure fish are rendered insensible—unable to perceive or respond to stimuli—prior to death and remain so without experiencing fear, pain, or distress. While electroencephalography (EEG) based neurophysiological indicators are the gold standard for assessing sensibility, their use requires specialist equipment and expertise, limiting feasibility in commercial settings.

As a result, operational indicators—such as the loss or recovery of equilibrium, ventilation, and the 'eye-roll' reflex—are commonly used on farms to assess sensibility. However, concerns remain regarding the time lag between these indicators and the actual cessation or recovery of brain activity. Additionally, these indicators may signify immobilization rather than true insensibility. Misjudging sensibility can compromise animal welfare during procedures such as exsanguination or evisceration.

To ensure their reliability, operational indicators must be validated against neurophysiological benchmarks on a species- and method-specific basis. Yet such validation is lacking for European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). This study addresses that gap by comparing operational and neurophysiological indicators during gradual (anaesthesia immersion) and rapid ('in-water' electrical stunning) transitions into and out of insensibility in both species, assessing the extent to which operational indicators reliably reflect true insensibility.

## Materials and methods

*Loss of indicators during gradual induction into insensibility*

To evaluate the gradual induction into insensibility in seabass (n = 16) and seabream (n = 14), fish were transferred to a container containing saltwater with an anaesthetic solution (salinity = 9.2 ‰, temperature = 24.0ºC, anaesthetic = 600 ppm 2-phenoxyethanol). Operational indicators (*e.g.*, loss of equilibrium, ventilation, and 'eye-roll' reflexes) and the loss of VERs were then monitored during the gradual induction into insensibility.

*Recovery of indicators following acute induction into insensibility*

To evaluate the recovery of seabass (n = 21) and seabream (n = 27) following acute induction into insensibility, fish were transferred to a darkened, in-water electrical stunning chamber containing saltwater (salinity = 9.2 ‰, temperature = 23.8ºC). Fish were then individually subjected to a 30 s electrical stun (*i.e.*, 50 Hz AC, current density of 3.04 A $dm^{-2}$, electrical field strength of 1.68 V $cm^{-1}$) and the recovery of operational indicators and VERs were then monitored

## Results and discussion

Until neurophysiological monitoring becomes feasible in commercial practice, detailed, species- and method-specific validations of operational indicators remain essential. This study highlights the limitations of operational indicators and underscores the critical need for robust, context-dependent assessments to safeguard fish welfare during slaughter in aquaculture.

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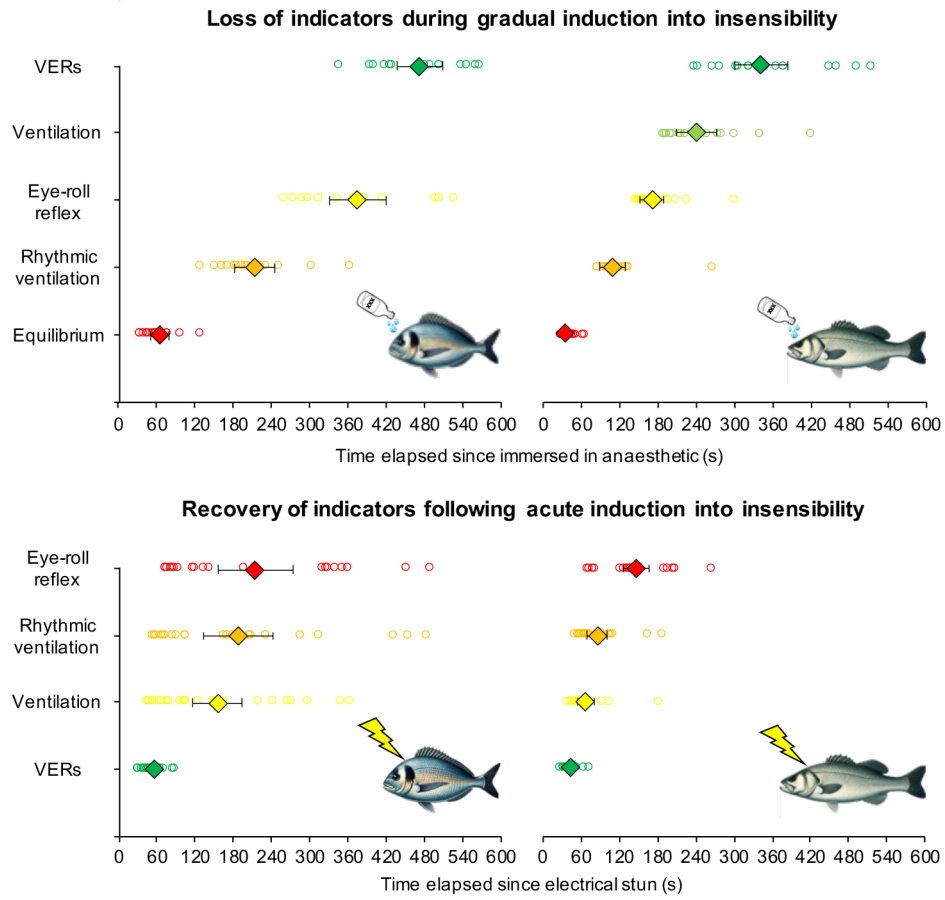


**Figure 1:** The relationship between operational and neurophysiological indicators during a gradual, or following an acute, induction into insensibility in seabass and seabream. Raw data points are represented by the unfilled coloured circles, while the mean ± 95% C.I. are represented by the filled coloured diamonds and error bars.

Our findings reveal significant temporal mismatches between operational and neurophysiological indicators of sensibility. During anaesthetic immersion, all operational indicators were consistently lost before VERs, with equilibrium lost first and ventilation last (see upper panel of Fig. 1). This mismatch ranged from 1.3 to 8.1 times faster than VER loss, depending on the indicator and species. Similarly, following electrical stunning, operational indicators reappeared substantially later than VERs, by factors ranging from 1.4 to 4.7 (see lower panel of Fig. 1). These findings confirm that an exclusive focus on the absence of operational indicators may lead to incorrect assumptions about the neural state of the individual. Clear interspecies differences also emerged. Seabass lost and regained sensibility faster than seabream across nearly all indicators. Interestingly, the sequence of indicator loss differed between species during anaesthesia, further supporting the need for species-specific welfare assessments.

Despite the rising use of electrical stunning, the majority of fish regained VERs within seconds to minutes following a 30 s ‘in-water’ electrical stun. In fact, more than half of individuals already displayed VERs by the time EEG recordings began, underlining how quickly sensibility can return. This rapid recovery poses a serious welfare hazard if not followed by prompt and effective killing. Consequently, there is an urgent need to rigorously evaluate, improve, and validate electrical stunning methods in seabass and seabream before promoting and implementing them as ‘humane’ stunning methods.

# EFFECTS OF DIETARY SOLUBILIZED MANNANOLIGOSACCHARIDES ON PERFORMANCE *Penaeus vannamei*, RAISED IN AN INTENSIVE SYNBIOTIC SYSTEM

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## Introduction

A new generation of MOS-based prebiotics is emerging that achieves solubilization of the mannan layer within the yeast cell wall, making it more accessible and partially exposing the glucan layer. This synergistic process enhances the overall efficacy of these prebiotics. β-Glucan is a complex polysaccharide that has been shown to have remarkable immunostimulatory, anti-inflammatory and antioxidant properties in aquatic animals. This study was conducted to evaluate the effects of dietary supplementation with commercially available solubilized mannanoligosaccharides (MOS) on performance, health status (gut bacterial and fungal counts, ammonia and nitrite stress, gut morphology), and economic benefits of Pacific white shrimp, *Penaeus vannamei* raised in an intensive synbiotic system.

## Material and methods

This diet was designed to be both isoproteic (360 g of protein per kg) and isoenergetic (4400 Kcal per kg). The commercial source of mannan oligosaccharides (MOS) used consists of 27.2% solubilized mannan oligosaccharides, 17% crude protein, 3.4% ash, and 8% water. In addition to the control diet, three experimental diets were developed based on the control formulation to include the commercial MOS source, replacing wheat flour at different inclusion levels: 1.0 g (0.27 g mannans), 2.0 g (0.54 g mannans), and 4.0 g (1.08 g mannans) of solubilized MOS per kg of feed. These experimental diets were designated M1.0, M2.0, and M4.0, respectively. Shrimp, initially weighing 3.00 ± 0.04 g at a density of 100 shrimp $m^{-2}$, were randomly distributed and fed four times a day for 60 days. The shrimp weight (n = 20) was monitored every 10 days to assess growth and adjust the amount of feed. On day 30 and at the end of the experimental period, all shrimp were counted, and the following parameters were calculated: final weight, feed conversion ratio (FCR), survival rate, and yield.

**Table 1.** Performance of *P. vannamei* juveniles fed control and test diets with commercially solubilized mannanoligosaccharides in a synbiotic system for 60 days.

| Variables | Treatments | | | |
|---|---|---|---|---|
| | C | M1.0 | M2.0 | M4.0 |
| Final weight (g) | 8.86 ± 0.13$^{c}$ | 10.87 ± 0.26$^{a}$ | 9.30 ± 0.13$^{b}$ | 8.39 ± 0.17$^{d}$ |
| Survival (%) | 90.50 ± 1.50$^{a}$ | 91.67 ± 5.51$^{a}$ | 93.00 ± 3.00$^{a}$ | 73.00 ± 2.00$^{b}$ |
| Growth week $g^{-1}$ | 0.68 ± 0.01$^{b}$ | 0.91 ± 0.03$^{a}$ | 0.73 ± 0.02$^{b}$ | 0.63 ± 0.02$^{c}$ |
| kg $m^{-3}$ | 1.0 ± 0.01$^{c}$ | 1.25 ± 0.09$^{a}$ | 1.08 ± 0.02$^{b}$ | 0.77 ± 0.02$^{d}$ |
| kg $ha^{-1}$ | 8,014 ± 131$^{c}$ | 9,967 ± 719$^{a}$ | 8,644 ± 161$^{b}$ | 6,182 ± 51$^{d}$ |
| FCR | 1.70 ± 0.01$^{b}$ | 1.20 ± 0.14$^{d}$ | 1.50 ± 0.02$^{c}$ | 2.10 ± 0.10$^{a}$ |

Data are expressed as mean (n=3) ± standard deviation. The results were analyzed with ANOVA ($p \leq 0.05$) followed by the Tukey test. Mean values on the same line with different superscripts differ significantly.

*(Continued on next page)*

**Results**

At the end of the 60-day experimental period, significant differences were observed in shrimp performance between the treatments (Table 1). Diets supplemented with solubilized MOS, particularly M1.0 and M2.0, showed better results compared to the control group. The M1.0 treatment exhibited the highest average final weight (10.87 ± 0.26 g), significantly higher ($p < 0.05$) than the control group (8.86 ± 0.13 g). This group also had a higher weekly growth rate (0.91 ± 0.03 g $week^{-1}$) and better productivity, (1.25 ± 0.09 kg $m^{-3}$, 9,967 ± 719 kg $ha^{-1}$), when compared to other treatments. The feed conversion ratio (FCR) was most efficient in the M1.0 treatment (1.20 ± 0.14), significantly lower than in the control and other MOS diets. Survival was also higher in the M1.0 and M2.0 diets (91.67% and 93.00%, respectively), while the lowest value was recorded in the M4.0 group (73.00%).

**Conclusion**

Supplementation with 1.0 g and 2.0g of commercially solubilized MOS $kg^{-1}$ in the diet of *P. vannamei* raised in an intensive synbiotic system significantly improved final weight, feed conversion ratio and weekly growth rate compared to the control.

# PROBIOTICS AND SYNBIOTICS IN THE NURSERY CULTURE OF *Penaeus vannamei*: EFFECTS ON GROWTH AND ECONOMIC BENEFITS

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## Introduction

The synbiotic system (fermentation of prebiotics + probiotics = synbiotics) represents an innovative microbial biotechnology approach for recovery and utilization of waste in aquaculture, as it contributes to water quality management (acceleration of nitrification processes, reduction of organic load, *Vibrio* decrease and settleable solids) and the development of healthy microbial communities within the system. The prebiotic compounds such as rice, soybean, or wheat bran serve as fermentable substrates that enhance the colonization capacity and activity of probiotics. The probiotics typically include active microbial strains such as *Bacillus*, *Bifidobacterium*, *Lactobacillus*, or yeast species like *Saccharomyces cerevisiae*, which, when administered in adequate amounts, confer health benefits to the host. Its application has shown promising results in both intensive and semi-intensive aquaculture operations, particularly those with minimal water exchange. In this context, studies assessing the productive and economic benefits of probiotic and synbiotic applications during the nursery phase are critically important. These approaches may contribute to the economic sustainability of shrimp aquaculture by reducing production costs and improving juvenile quality. Therefore, this study aims to evaluate the effects of probiotic and synbiotic application strategies on water quality, presumptive microbial counts, and economic performance during the nursery phase of *Penaeus vannamei*.

## Material and methods

The shrimp with an average weight of 0.010 ± 0.001 g were transferred to 16 experimental tanks at a density of 280 PLs per tank (5,000 PLs $m^{-3}$) and cultured for 30 days. A completely randomized design was used, consisting of four treatments with four replicates each: Control (C): no probiotics or synbiotics; Probiotic (P): application of commercial probiotics strains activated with demerara sugar (2 hours); Synbiotic (S): application of rice bran fermented with commercial probiotic strains; Probiotic + Synbiotic (SP): alternating application of activated probiotics strains activated with demerara sugar (2 hours) and fermented rice bran containing probiotics. The experimental system consisted of 16 rectangular tanks with an operational volume of 56 L each. Before stocking, all tanks were filled with 50 L of seawater treated with sodium hypochlorite at 40 g $m^{-3}$ of active chlorine. Then, 6 L of inoculum were added from water an active shrimp nursery with the following physicochemical characteristics: total ammonia nitrogen (TAN) = 0.06 mg $L^{-1}$, nitrite-N = 0.03 mg $L^{-1}$, nitrate-N = 244.5 mg $L^{-1}$, total alkalinity = 140 mg $CaCO_3$ $L^{-1}$, pH = 8.0, settleable solids = 4.8 mL $L^{-1}$, orthophosphate = 23.6 mg $L^{-1}$, and salinity = 24.9 g $L^{-1}$. The synbiotic was prepared using a mixture of rice bran (as a carbon organic source), demerara sugar (also a C source), a commercial probiotic blend (*Bacillus subtilis* and *Bacillus licheniformis*, $5 \times 10^{10}$ CFU $g^{-1}$, PRO-W®, INVE Aquaculture Inc.), and sterilized water. After homogenization, the mixture underwent anaerobic fermentation for 24 hours followed by 24 hours of aerobic fermentation prior to use. At the end of the 30-day trial, shrimp performance was evaluated using the following parameters, calculated based on the surviving shrimp: Biomass gain (g) = Final biomass (g) – Initial biomass (g); Final weight (g) = Final biomass (g) / Number of surviving individuals; Feed Conversion Ratio (FCR) = Feed offered (g) / Biomass gain (g); Survival rate (%) = (Final number of shrimp / Initial number) × 100; Specific Growth Rate (SGR, % $day^{-1}$) = 100 × [(ln Final weight – ln Initial weight) / 30]; Yield (kg $m^{-3}$) = Final biomass (kg) / Tank volume ($m^3$). Input costs and shrimp performance parameters (final weight, productivity, and feed conversion ratio) were extrapolated to a 100 $m^3$ nursery tank to estimate the economic benefits of each treatment. Cost estimates for inputs were based on the following values: USD 72.02 per kg of probiotic; USD 0.58 per kg of synbiotic; USD 14.90 per kg of nursery feed; and USD 2.31 per 1,000 post-larvae. Economic benefits were calculated using the formulas: Feed cost (CR) = biomass gain × FCR × feed price per kg; Probiotic cost (CP) = 1 g/$m^3$ × 100 $m^3$ × 6 applications × probiotic price/kg; Synbiotic cost (CS) = 10 g/$m^3$ × 100 $m^3$ × 6 applications × synbiotic price/kg; PL cost (CPL) = 5,000 PL/$m^3$ × 100 $m^3$ × PL unit price; Total cost (CT) = (CR + CP + CS + CPL) / survival; Production cost difference (DCP) = control – treatment; Relative difference (DR) = DCP / control × 100.

*(Continued on next page)*

Table 1. Growth performance of *P. vannamei* in nursery phase with probiotic and/or synbiotic supplementation.

| Variables | Treatments | | | |
|---|---|---|---|---|
| | C | P | SP | S |
| Final weight (g) | 0.57±0.06[a] | 0.52±0.04[a] | 0.52±0.04[a] | 0.52±0.05[a] |
| Survival (%) | 82.57±2.81[b] | 94.47±5.50[a] | 97.12±4.05[a] | 98.22±3.55[a] |
| FCR | 1.26±0.11[a] | 1.22±0.09[a] | 1.19±0.05[a] | 1.17±0.07[a] |
| SGR (% day$^{-1}$) | 13.46±0.35[a] | 13.16±0.27[a] | 13.15±0.31[a] | 13.15±0.33[a] |
| Yield (kg m$^{-3}$) | 2.38±0.20[a] | 2.45±0.014[a] | 2.50±0.11[a] | 2.54±0.17[a] |

Data are expressed as mean ± standard deviation (n = 4). Different superscript letters in the same row indicate significant differences ($p < 0.05$).

**Results**

Supplementation with probiotic strains, with or without fermentation, significantly improved shrimp survival after 30 days. However, no significant differences ($p > 0.05$) were found for final weight, specific growth rate (SGR), feed conversion ratio (FCR), or yield (Table 1). Economic gains from probiotic and synbiotic supplementation, based on projections for a 100 m$^3$ nursery tank, ranged from USD 700 to USD 978, with relative cost reductions of 10–14% compared to the control (Table 2).

**Conclusion**

In this study, survival at the end of the nursery phase was significantly improved by *Bacillus* applications. The use of 1 g m$^{-3}$ ($10^{10}$ CFU g$^{-1}$) every five days enhanced and yielded economic benefits (increased revenue and relative cost difference) compared to the control. However, the greatest economic benefits were achieved with synbiotic applications.

# EFFECT OF MICROALGAE AND PROBIOTIC ENRICHED BIOFLOC ON STRESS RESPONSES OF *Mugil cephalus* UNDER OXYGEN AND DENSITY CHALLENGES

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**Introduction**

One of the major challenges in aquaculture is ensuring the sustainable expansion of the sector. In this context, several efforts have been directed toward investigating the application of Biofloc Technology (BFT) for a range of species. BFT is an example of sustainable aquaculture due to minimal water exchange and for enabling the recycling of nutrients, which are converted into bacterial protein (bioflocs). The rearing of omnivorous species in bioflocs has shown promising results, mainly due to the ability of these animals to take advantage of the biofloc as a complementary food source. Furthermore, research on *Mugil cephalus*, a native species of the Mediterranean and one of the promising candidates for aquaculture among omnivorous fish in this region, has also demonstrated that it can adapt well to biofloc. Thus, as BFT is a closed system, the introduction of functional additives in the water can be an interesting way to further enrich the system and, potentially, enhance the immune responses of the animals, for instance, through the addition of microalgae and probiotics. Therefore, the objective of the present study was to evaluate the effect of microalgae *Chlorella* and probiotic *Bacillus* addition on stress responses under oxygen and density challenge.

**Material and Methods**

For the experiment, 180 animals with an initial weight of 91,7 ± 22,4 grams were distributed in 12 circular tanks filled with 800 L of water at a salinity of 20 (10% of water was composed of a mature biofloc inoculum). The trial included 4 treatments in triplicate: microalgae addition (Microalgae), probiotics addition (Dark+Prob), microalgae and probiotics addition (Micro+Prob), and a control, just biofloc (Dark). The light restriction was used in Dark and Dark+Prob treatments to establish a heterotrophic system, while in the Microalgae and Micro+Prob treatments, a 12:12h photoperiod was used to maintain the microalgae and a mixotrophic system. The probiotic (Sanolife PRO-W *Bacillus subtilis* and *B. licheniformis*) and the microalgae (*Chlorella vulgaris*) were added to the corresponding treatments once a week, at 1g/$m^3$ and 6L/tank at 1A (corresponding to 18 x $10^6$ cells/ml), respectively. A commercial feed (DIBAQ - 3.5 mm) with 30% crude protein and 7% crude fat was provided three times daily at a 2 %/d feeding rate. The trial lasted 41 days. At the end of this period, the animals were exposed to a 4-hour stress challenge, followed by 24 hours of recovery, when the parameters were restored to standard levels. For the stress challenge, the animals were kept in a condition of Hypoxia (dissolved oxygen 2mg/L) + High density (50 kg/$m^3$). Before the stress, immediately after it, and at the end of the 24-hour recovery period, blood and liver samples were collected to analyze cortisol and enzymes of oxidative stress (n=2 animals per tank/time). Results were analyzed using a repeated measures ANOVA, considering the factors treatment and sampling time. Mauchly's test of sphericity was applied, and significant differences were verified by applying Fisher's LSD test at a 95% significance level ($p < 0.05$).

**Results and discussion**

At the end of the 41-day experimental period, the animals showed 100% survival in all treatments. Similarly, the stress challenge did not result in mortality. Regarding oxidative stress enzymes, the hypoxia and high-density challenge did not alter significantly the activity of the superoxide dismutase (SOD), although, among the groups, the Micro+Prob treatment was the one with the highest initial activity (0h). In contrast, the enzyme Catalase (CAT) was significantly reduced in all groups, except microalgae. After 24 hours of recovery, only the Dark group showed no restoration in CAT activity, which remained significantly lower than the Micro+Prob group. The stress condition increases glutathione peroxidase (GPx) activity in all groups. Only the Microalgae group demonstrated recovery of enzymatic activity to levels comparable to the basal condition (0h). With respect to cortisol, the challenge led to a significant increase in this parameter in all groups. Although the addition of microalgae and probiotics had a protective effect compared to the control, there was a lower increase in cortisol.

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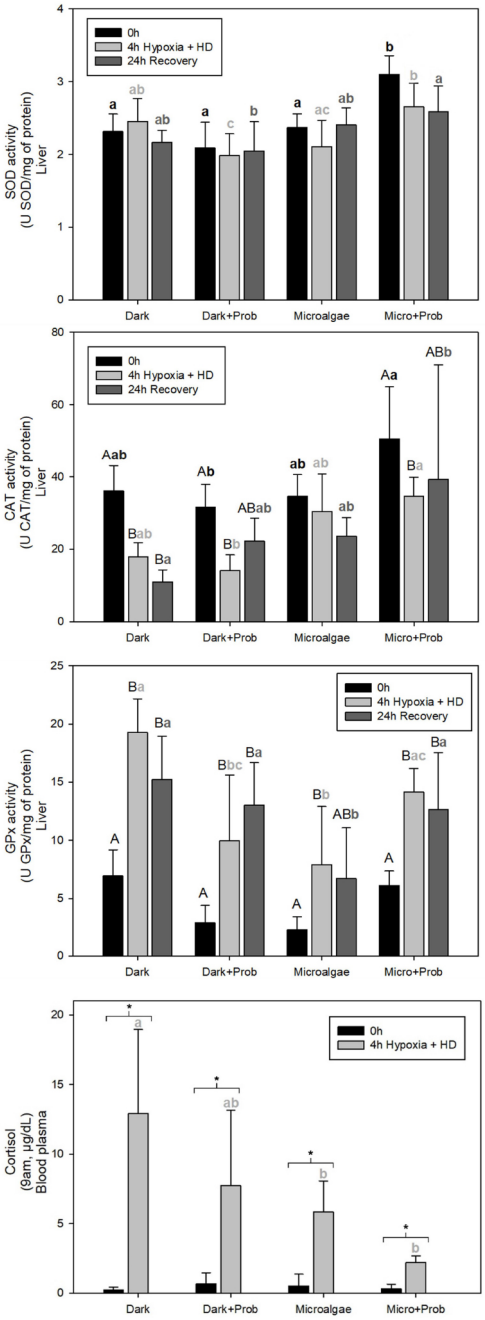


**Fig 1.** Activity of antioxidant enzymes in the liver of *Mugil cephalus*. (a) SOD, (b) CAT, (c) GPx and Cortisol level in the blood plasma (d). Different uppercase letters indicate significant differences among sampling times within the same treatment, and lowercase letters indicate significant differences between treatments at the same time point. For cortisol levels, '*' indicates significant differences within groups. A level of 5% ($p < 0.05$) was used for all analyses. Data are expressed as mean ± standard error (n=6).

There are few studies available on the inoculation of microalgae directly into biofloc and the associated oxidative stress response. On the other hand, many studies using *C. vulgaris* supplemented in fish diets report an improvement in the antioxidant capacity of the animals, mitigating the effects caused by oxidative stress. These findings agree with the results found in this study. One of the arguments behind the protective effect of *C. vulgaris* is related to pigments, such as carotenoids, which function against the reactive oxygen species generated under stressful conditions, performing a scavenging role. The use of probiotics is well established in BFT, especially in marine shrimp farming. The gender *Bacillus* is one of the most used in aquaculture, improving immunity and health status. Considering our results, the combination of the probiotic with microalgae led to improvements in the analyzed parameters when compared to the probiotic-only group. This was evidenced by a more effective antioxidant enzyme activity and a reduced increase in cortisol levels. In conclusion, combining microalgae and probiotics into the BFT demonstrates significant potential and an effective strategy to improve animal health in response to stress conditions.

**Acknowledgments**

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# COMPARATIVE BENCHMARKING OF ELECTRIC VS. DIESEL AERATION IN SALMON AQUACULTURE

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Aeration is a critical intervention in open-net-pen salmon aquaculture, used to maintain water quality and support fish welfare during periods of environmental stress. Conventional diesel-powered aeration systems, while effective, are energy-intensive, costly, and contribute significantly to greenhouse gas emissions. Here, we evaluate a novel electric-powered smart aeration unit (Pneuma) as a low-cost, energy-efficient, and environmentally sustainable alternative.

A trial was conducted at a commercial salmon net-pen site in Scotland to benchmark the performance of the Pneuma system against a standard diesel compressor, both running continuously. Over a two-week period in November 2024, two pens were aerated using the two systems, with air diffusers deployed at 7m depth. The trial aimed to assess operational efficiency, air delivery, and environmental impact in commercial aquaculture conditions.

Water quality was monitored using sensors deployed at multiple depths and locations within each pen capturing high-resolution data on dissolved oxygen, temperature, salinity, and chlorophyll. Power consumption and air output were recorded using inline airflow meters and power monitors, and diesel consumption was recorded manually. Additional environmental data, including tidal regime, turbidity and weather conditions, were also collected to contextualise system performance.

The trial provides a robust dataset for comparing the operational efficiency and environmental footprint of electric versus diesel aeration systems. The findings will inform a life-cycle assessment of the two systems. Building on this work, a second trial will run over 12 months to evaluate the impact of on-demand electric aeration on fish health, welfare, and performance compared to conventional diesel aeration. Outputs from this project will support the development of sustainable, scalable aeration strategies for the salmon aquaculture sector.

# EFFECT OF OREGANO ESSENTIAL OIL AND QUILLAJA SAPONIN EXTRACT ON THE POLAR TUBE EXTRUSION ACTIVITY OF SHRIMP MICROSPORIDIAN *Enterocytozoon hepatopenasei*

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## Introduction

Oregano essential oil (OEO) has been widely shown to support the health and productivity of various production species. Orego-Stim Forte (Anpario plc) is a commercial additive containing OEO and *Quillaja saponaria* extract. The trial objective was to determine whether this feed ingredient can inhibit the polar tube extrusion of *Enterocytozoon hepatopenasei* (EHP) spores that infect shrimp and cause significant economic losses to production.

## Materials and Methods

The study consisted of two phases. Phase one aimed to determine the inhibitory effect of the test ingredient on the polar tube extrusion activity of EHP spores. Concentrations ranged from 500 to 4000ppm with 30 minute contact time. For phase two, the infectivity of ingredient-treated EHP spores was tested following oral intubation of live shrimp. The SWP-qPCR method of hepatopancreas samples was used to determine the EHP replication levels of those spores fed orally to shrimp after 9-10 days.

## Results

For both low and high-salinity sourced EHP spores, no polar tube extrusion was detected when exposed to ≥2000ppm of the test ingredient (Figure 1).

Second phase results were collected following oral intubation of shrimp with ingredient-treated EHP spores from the first phase of the trial. SWP-qPCR of hepatopancreas samples revealed no infectivity of treated EHP spores when exposed to ≥2000ppm and ≥1000ppm concentrations from low and high salinity sources respectively.

## Summary

The test ingredient at ≥2,000ppm, containing OEO and quillaja saponin extract, inhibited polar tube extrusion of EHP spores *in-vitro*. Oral inoculation of treated spores at ≥2,000ppm confirmed inactivity since no replication was detected following SWP-qPCR of shrimp hepatopancreas samples 9-10 days post-injection.

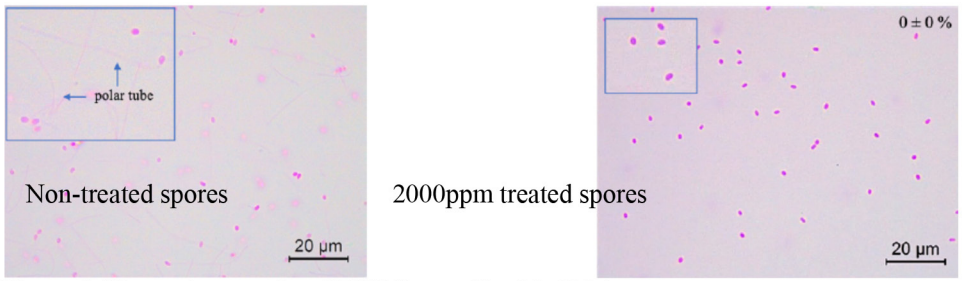


Figure 1. Photomicrographs of EHP Spores Used in Trial

# UNLOCKING COMMERCIAL SCALE GENOME EDITING

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Genome editing technologies, particularly those utilizing Site-Directed Nuclease 1 (SDN-1), are poised to transform aquaculture by enabling precise, efficient modifications to traits critical for productivity and sustainability. Traits such as sterility, accelerated growth, and yield can now be enhanced at scale in a single generation, offering clear commercial advantages. Recent breakthroughs in high-throughput genome editing—including somatic cell editing efficiencies exceeding 95% and scalable production of sterile fish—are driving the transition from research to industrial application. However, the path to global adoption is shaped not only by technical success, but by regulatory readiness.

The regulatory status of genome-edited animals, particularly those generated using SDN-1 approaches that do not introduce foreign DNA, varies widely across jurisdictions. While some countries have opted not to regulate SDN-1 organisms as genetically modified, others maintain strict oversight or lack clear guidance—posing potential barriers to commercialization and international trade. For export-oriented aquaculture producers, the regulation of the products of genome editing can directly impact market access and return on investment.

This presentation synthesizes current technical progress in commercial genome editing with emerging regulatory frameworks, and explores their implications for industry adoption, breeding program integration, and global trade. It emphasizes the urgent need for international regulatory alignment to unlock the full potential of genome editing and ensure that innovations translate into sustainable growth and competitiveness in aquaculture.

# SKIN TRANSCRIPTOMIC ANALYSIS IN ATLANTIC SALMON, EUROPEAN SEABASS, AND RAINBOW TROUT UNDER TRANSPORT-INDUCED ACUTE STRESS

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**Introduction**

Fish transport is a widely used and unavoidable essential practice in aquaculture for purposes such as stocking, breeding, and growth. However, this process often exposes fish to acute stress, which can significantly affect their physiology and overall welfare. The severity of transport-induced stress can vary depending on the initial health status of the fish and transportation conditions, including duration, stocking density, temperature, water quality, and ammonia concentration. While transport rarely causes immediate mortality, secondary and tertiary stress-related responses can compromise both specific and nonspecific immunity, increasing susceptibility to disease and osmoregulatory imbalances, which may lead to mortality. Current methods for assessing immune and health status rely on invasive procedures (e.g., blood and tissue collection). Established welfare indicators, which help assess how well fish needs are met, are often observed too late to enable timely intervention, and remain limited for many farmed species. Further research is needed to understand how common aquaculture practices, such as transport, impact farmed fish.

**Experimental design and methodology**

To investigate the molecular signatures following acute stress, three key European aquaculture species – Atlantic salmon (*Salmo salar*, AS), European seabass (*Dicentrarchus labrax*, ES), and rainbow trout (*Oncorhynchus mykiss*, RT) were submitted to transport induced stress. The transportation trials were designed to simulate current transport conditions specific to each species. Skin tissue samples were collected at three time points: before transport, immediately after stress exposure, and following a 24-hour recovery period. RNA sequencing (RNA-seq) followed by differential gene expression (DGE) analysis and functional enrichment were performed to examine transcriptomic responses.

**Results and future prospects**

A comparative analysis across species revealed shared molecular pathways: mitogen-activated protein kinase (MAPK) signaling pathway was enriched in all three species, and ErbB and FoxO signaling pathways were enriched in AS and RT. A set of common differentially expressed genes was also observed, including 36 that were upregulated in response to stress. Among the upregulated genes are several involved in key biological functions such as immunity, apoptosis, stress modulation and epithelial regeneration. These findings offer valuable insights into conserved stress response mechanisms. Ongoing research will incorporate proteomic analysis of skin mucus and miRNA profiling from water samples to provide a more comprehensive understanding of the underlying biological processes and to support the identification of potential biomarkers for use in minimally invasive biosensors.

**Acknowledgements**

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# EARLY-LIFE MICROBIAL DYNAMICS IN BARRAMUNDI HATCHERIES REVEALED BY FLOW CYTOMETRY AND 16S PROFILING

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Barramundi (*Lates calcarifer*), native to the Indo-Pacific, has rapidly become the fastest-growing aquaculture species worldwide. However, maintaining stable production during the larviculture stage remains challenging, with larval mortality often reaching 100%. As hatchery management practices play a crucial role in regulating water microbiota, understanding microbiome dynamics is essential for improving survival rates. From day post hatch (DPH) 0 to DPH 17 at a commercial barramundi larviculture facility in Vietnam, microbial dynamics and community assembly in rearing tanks were analyzed using flow cytometry (FCM) and 16S rRNA gene amplicon sequencing. The analysis revealed persistent tank-specific differences in microbial communities that fluctuated over time. Stochastic factors played a significant role in microbial assembly, leading to variability between tanks even under similar operational conditions and underscoring the complexity of microbial community dynamics in larviculture systems. Flow cytometry and 16S rRNA sequencing showed moderately correlated diversity metrics and captured comparable overall trends in community composition, reflecting their potential complementarity in monitoring microbial dynamics. This underscores the potential of FCM as a complementary, rapid, and cost-effective approach for monitoring microbial community dynamic in aquaculture.

# DOES THE EMBRYONIC ENVIRONMENT IMPACT SMOLTIFICATION OF ATLANTIC SALMON (*Salmo salar*)?

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## Introduction

The Atlantic salmon industry is challenged by large losses at sea, especially during the first months after the transfer from freshwater to seawater, which is often attributed to a poor smoltification status of the fish. Although a vast array of research is currently directed towards the understanding of the process of smoltification, alternative solutions to the problem may lie with manipulations of the early embryonic environment. It has been shown that the embryonic environment plays a crucial role in the performance of fish later in life. For example, temperature during early life appears to influence growth and immune function. Here we explore whether embryonic temperature impacts the smoltification process of Atlantic salmon.

## Materials and methods

Atlantic salmon embryos were exposed to different temperatures (4C and 8C) between fertilization and the 'eyed-stage'. Afterwards, similar temperature profiles have been applied. Smoltification was induced using a standard photoperiod regime. At consecutive time points gene expression levels of smoltification and oxidative stress markers were analysed in, respectively, gill and liver tissue. Hepatocytes of postsmolts were isolated and exposed to various stressors. Furthermore, blood serum metabolomics are currently being analysed.

## Results

Our preliminary results show that performance with respect to growth was not significantly impacted by embryonic temperature. Whereas the expression levels of the smoltification markers *nka1a* and *nka1b* in the gill showed similar patterns between the two temperature groups, *s100a* and *cftr2* showed opposite patterns. The oxidative stress marker *gpx* increased in the low temperature group, while *cat* and *cu/znsod* decreased over time in the 8C group with respect to the 4C group. Hepatocytes showed little differences between the embryonic temperature groups in their response towards hydrogen peroxide, polyIC or LPS.

## Conclusion

In order to improve the robustness of Atlantic salmon during its later life phases, the embryonic environment should be taken into consideration. It appears that the embryonic temperature may influence smoltification.

## Acknowledgements

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# EFFECT OF FISHPOND MANAGEMENT ON BIOTIC COMMUNITIES, PHYSICOCHEMICAL CHARACTERISTICS OF THE ENVIRONMENT AND COMMON CARP (*Cyprinus carpio*) FOOD SOURCE PREFERENCES AND MOVEMENT ECOLOGY

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**Introduction**

Fishponds are essential ecosystems inhabited by many different species and serving commercial and non-commercial functions. Therefore, the management of fishponds can be a crucial factor affecting all the organisms inhabiting a given pond, as well as the physicochemical parameters of the pond water.

In this study, we investigated the effects of traditional (business as usual, BAU) and adjusted fishpond management (natural production, NP; natural production with supplementary feeding, NPF) on biota diversity and its seasonal development (macrozoobenthos, zooplankton and phytoplankton), the development of physicochemical water parameters, and the food source preferences and movement ecology of common carp.

**Materials and Methods**

To evaluate the impact of above-mentioned management strategies, four ponds were monitored monthly throughout the vegetation season over a period of three years (2021–2023). At each sampling point, the entire biota of the fishpond (fish, macrozoobenthos, zooplankton and phytoplankton) was sampled. In addition, physicochemical water parameters were monitored over the same period. To reveal the movement ecology of common carp, acoustic telemetry was employed. Additionally, stable isotopes and a fatty acid approach were employed to reveal the common carp's feeding preferences. The management of fish stocks was based on the natural production of the given ponds and the level of supplementary feeding planned.

**Results**

***Biotic communities*:** Fishpond management significantly impacted the seasonal development of the macrozoobenthos, zooplankton, and phytoplankton communities. Macrozoobenthos communities were the most sensitive to differences among BAU, NP and NPF, while zooplankton and phytoplankton communities were less sensitive.

***Water physicochemical parameters:*** Fishpond management had a significant impact on the physicochemical parameters of the water. In addition, the seasonal development differed between BAU, NP and NPF management. The most important of parameters affected by pond management were dissolved inorganic nitrogen, chlorophyll a, and total phosphorus.

***Common carp food source preferences**:* The preferred food sources changed over the season, regardless of the type of pond management. In NP management, common carp simply follow the most abundant food source. Nevertheless, when supplementary feeding was applied, the common carp utilized it at a higher rate, regardless of the abundance and biomass of other potential food sources.

***Common carp movement ecology*:** The activity and spatial use of carp changed over time, influenced by temperature, food availability, and stocking density. Their activity peaked in spring and early summer, with the highest levels occurring at night and dusk. During warmer months, the fish favored shallow areas (less than 0.5 meters deep), probably due to thermoregulation, oxygen levels or access to prey. Variability in the use of foraging sites and food composition among individuals correlated with growth, highlighting the importance of behavioral traits in resource acquisition.

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**Discussions**

This study highlights the complex interactions between fishery management practices and the aquatic communities of Central and Eastern European carp ponds. The management of fishponds is a key factor influencing the communities that inhabit them, as well as the physicochemical parameters of the water, the movement ecology of common carp and their food source preferences.

While the ponds may appear similar, the combination of environmental and human-induced factors has caused them to evolve in slightly different ways. Fish stocking based on the pond's natural production and proper supplementary feeding (here calculated to achieve a net production of 220 kg/ha of carp from additional feeding) does not negatively affect the communities and temporal trends of zooplankton and macrozoobenthos.

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# USING A MACHINE LEARNING ALGORITHM TO ASSESS ATLANTIC SALMON (*Salmo salar*) BEHAVIOUR IN RELATION TO GILL HEALTH IN COMMERCIAL AQUACULTURE FARMS

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## Introduction

Aquaculture continues its global expansion, reaching a record production of 130.9 million tonnes in 2022, valued at USD 312.8 billion (FAO, 2024). As the industry grows, more sophisticated technology is needed to monitor farms and ensure their sustainability. Using behaviour as a non-invasive monitoring tool, particularly when combined with artificial intelligence (AI) and machine learning, offers new opportunities for real-time farm management and improved welfare outcomes (Feng et al., 2024). The growing adoption of computer vision has further enabled the use of cameras and image-based systems with algorithms to extract and analyse behavioural data in a non-invasive and cost-effective manner (Georgopoulou et al., 2021). These technologies allow for continuous observation of fish, providing insight into group dynamics in commercial farms that are otherwise difficult to monitor.

Building on a preliminary study conducted in 2023 (Burke et al., 2025; Burke et al., *in prep*), which identified behavioural changes associated with poor gill health in Atlantic salmon across 2 Scottish farms, the present study aims to further investigate the relationship between group behaviour and gill health in commercial sea cages. This expanded research includes additional sites across Scotland and integrates a broader range of welfare indicators to corroborate these past findings. It also explores potential contributing factors to gill health issues (e.g. environmental parameters, harmful plankton, micro-jellyfish) to better understand variation in onset across cages and farms.

## Methods

In the preliminary study, cameras were deployed at 2 Scottish Atlantic salmon farms. Cages at each farm had a camera used for feeding located in the centre of the cage at mid-depth. An algorithm was created by Observe Technologies to process video footage from these cameras and transform it into behavioural data, termed 'activity', which encompasses fish abundance, speed, and shoal cohesion. Daily validation occurred for the duration of the study, whereby experts compare the videos to output from the algorithm. Additionally, gill health scores were evaluated weekly at both sites through the visual sampling of 10 fish in each cage (0 = healthy gills, 5 = necrotic tissue). During the summer 2023, gill health issues arose at both farms, leading to fish stress which was evident in the behavioural data. For the present study, cameras with the algorithm were deployed at 9 additional farms which consisted of between 10-14 stocked cages. Along with the gill health scores recorded by the farmers, the AGD histopathology results, operational welfare indicators, feeding and environmental parameters (e.g. temperature, oxygen, plankton, jellyfish) will be analysed.

## Results and Discussion

Preliminary findings from an earlier phase of this project demonstrated a potential relationship between group-level behavioural patterns (activity) and gill health in Atlantic salmon (Fig. 1). This as also associated with decreased feeding rate and increased mortality.

The current study expands on this by incorporating additional sites. It is anticipated that consistent changes in behavioural metrics such as activity and spatial cohesion will be observed in association with deteriorating gill condition. If confirmed, these findings will support the development of real-time, non-invasive welfare indicators. Final results will be available for presentation and discussion at the conference.

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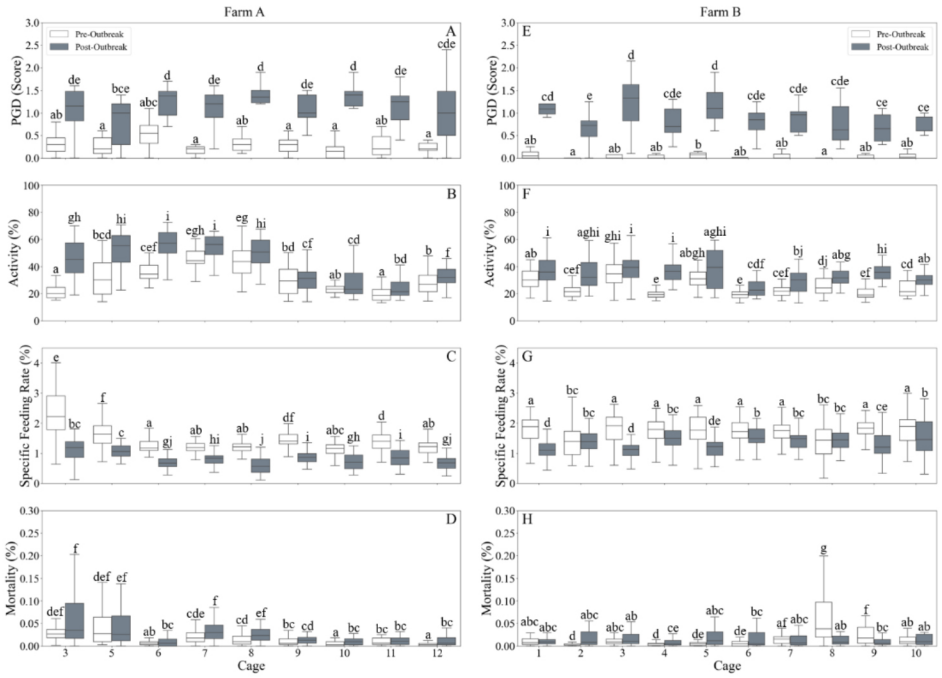


Figure 4 The health data from each cage in Farm A (left) and Farm B (right) including: The weekly PGD scores (A. E), the daily activity of the fish (B, F), the daily specific feeding rate (SFR; C, G), and the daily mortality (D, H). The colours indicate average 2 months pre- (white) and post- (grey) increase in activity (July 7 [Farm A]; August 18 [Farm B]). Different letters denote significant differences within and among cages per farm.

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# INTEGRATION OF FUNCTIONAL ANNOTATION DATA INTO GENOMIC PREDICTION MODELS FOR VNN RESISTANCE IN EUROPEAN SEA BASS USING BAYESIAN AND MACHINE LEARNING APPROACHES

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**Introduction**
Diseases continue to be a major challenge for the European sea bass industry in the Mediterranean region. While traditional selective breeding for disease resistance based on estimated breeding value is often costly and complex, genomic selection utilising high-density SNP genotyping combined with functional annotation data offers a more efficient approach to improve genetic resistance that supports sustainable aquaculture across multiple generations and diverse populations. The aim of this study was to evaluate whether integrating functional annotation data into genomic prediction models enhances the accuracy of predicting resistance to viral nervous necrosis (VNN) in European sea bass (*Dicentrarchus labrax*), using both Bayesian and machine learning methods.

**Material and methods**
A 29-day VNN challenge test was performed on 990 juvenile European sea bass (6-20g) and mortality was recorded as a binary trait. The experimental fish were genotyped using the Med-Fish SNP array (27,740 SNPs), while the parents and 40 experimental fish were whole-genome sequenced (6,072,853 SNPs), allowing whole-genome imputation for all experimental fish.

SNP filtering was based on: (1) predicted regulatory regions identified by ATAC and ChIP-seq data in different tissues; (2) Ensembl annotations of open chromatin, promoter and enhancer regions; and (3) classification of coding and non-coding variants (including intergenic, intronic, upstream, downstream, 5' UTR, 3' UTR, and synonymous variants) using the Ensembl Variant Effect Predictor. The control dataset included SNPs that were located in quiescent regions in all tissues and were not categorised into one of the Ensembl functional categories. All analyses were performed both excluding and including chromosome 3, which harbors a major QTL for VNN resistance.

Genomic prediction of genetic merit for mortality was performed using Bayesian threshold models as implemented in GIBBSf90+/BLUPf90+. Each analysis was repeated 100 times, with 2000 SNPs randomly selected per repetition. To mimic a realistic genomic selection scenario, where the prediction equations are trained on a reference population that is distantly related to the selection candidates, prediction accuracy was assessed using two-fold cross-validation. The folds were constructed to minimise relatedness between clusters and maximise relatedness within clusters.

Machine learning methods, including extreme gradient boosting (XGBoost), COMBI, deepCOMBI and Chaos Game Representation (CGR), were also tested to gain insight into their performance in classifying the mortality phenotype. In this case, three datasets were created based on predicted regulatory regions identified from ATAC and ChIP-seq data: regions active in at least 80% of tissues (Active80), 50% of tissues (Active50) and 10% of tissues (Active10). The same two-fold cross-validation strategy was used to assess model accuracy.

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**Results and discussion**

Results showed significantly improved performance when SNPs in active regulatory regions, or in open chromatin, enhancer, and promoter regions, were combined with causal variants, with prediction accuracy increasing by up to 20.8% compared to the control dataset. In contrast, when causal variants were excluded, the results were more variable, ranging from a 12.6% decrease to a 15.1% increase in prediction accuracy. Better performance compared to the control dataset was observed when using coding and non-coding variants, with a 4–6% increase in prediction accuracy when causal variants were not included, and a 20–23% increase when causal variants were included.

Machine and deep-learning methods showed variable performance across functional datasets. Higher F1-scores were observed with Active50 and Active10 sets than those obtained with the control dataset (0.66-0.74 vs 0.42-0.62 for Active50, 0.64-0.76 vs 0.61-0.74 for Active10). CGR demonstrated efficient discriminative capability between mortality categories using the active datasets.

These findings underscore the value of functional annotation data in enhancing genomic prediction accuracy for VNN resistance. More broadly, the results suggest that functional annotation data can improve the robustness and transferability of genomic prediction models for complex economic traits in aquaculture. By leveraging biologically relevant SNPs, breeding programs can increase prediction accuracy across generations and genetically diverse populations, ultimately supporting more efficient and sustainable genetic improvement in fish farming.

**Acknowledgments**

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# BIOACTIVITY OF METAL-BASED NANOPARTICLES IN AQUATIC ORGANISMS: A MULTISCALE APPROACH

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Metal-based nanoparticles (NPs), including quantum dots (QDs) and rare earth metal (REM)-based nanomaterials, are increasingly utilized in biomedical and environmental applications due to their unique physicochemical properties. QDs—typically composed of semiconductor materials such as CdSe/ZnS—exhibit exceptional fluorescence, making them ideal for cellular imaging and biosensing. Similarly, REM-based NPs, including those doped with europium ($Eu^{3+}$) or containing cerium ($Ce^{4+}$), are valued for their luminescent, redox, and catalytic behavior. Nonetheless, increasing attention is being directed toward the potential nanotoxicity and long-term environmental persistence of NPs, particularly due to their adverse effects on aquatic organisms. These concerns highlight the need for multiscale evaluation frameworks that consider NP morphology, concentration, and physicochemical interactions with aquatic organisms, including algae, crustaceans, and fish. Our results demonstrate that QDs can induce changes in the gut microbiota of *Salmo trutta fario* juveniles, inhibit the growth of the green algae *Desmodesmus communis*, and affect the heart rate of *Daphnia magna*. Furthermore, the effects of rare earth metal (REM)-based NPs on *D. communis* were dependent on the algal growth medium, while their impact on *D. magna* varied with NP concentration. As nanotechnology continues to advance across various sectors, further research is essential to balance innovation with environmental and biological safety, ensuring the responsible and sustainable application of nanoparticles.

**Acknowledgement**
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# ORAL AND INTRAPERITONEAL VACCINATION AGAINST *Aeromonas salmonicida* PROTECTS SOLE TRIGGERING DIFFERENT IMMUNE MECHANISMS

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## Introduction

*Solea senegalensis* is one of the most thriving species of marine large-scale culture in Europe and one of the threats that causes important economic losses is the infection by *Aeromonas salmonicida*. Although there are different types of vaccines produced for aquaculture, the majority that are used for bacterial pathogens are killed whole-cell preparations. Besides protection, vaccine delivery is an area where significant improvement is needed. Fish vaccination is mainly conducted by injection (intraperitoneal or intramuscular), soaking (bathing or spraying), or oral routes (1). Oral vaccination is highly pursued in aquaculture as is a cost-effective, and almost effortless delivery method, suitable for all stages of development (2, 3). Their efficacy is dependent on antigen content in the feed, resistance against gastric degradation and antigen adsorption/uptake in the gut. Oral vaccines have also the advantage of facilitating the implementation of a vaccination regime with boosters that avoid fish handling and stress. Here, we developed two experimental vaccines, including oral and intraperitoneal (ip.) formulations, and determine the capacity of the new vaccine formulations to provide protection against *A. salmonicida* in the sole compared with the standard ip. autogenous vaccine used currently.

## Material and methods

Two oral formulations with whole or particulate antigen (OV-W/OV-P) at two different doses (high-HD, low-LD), and an intraperitoneal vaccine consisting of a gel adjuvant emulsion with liposomes formulated also with whole or particulate antigen (LIPV-W/LIPV-P) were administered to cultured soles. Their effects were compared to the standard ip. vaccine (STV) formulated with and oil-based adjuvant. The efficacy of the vaccines was analyzed by challenging sole at 90-days post-vaccination by ip. inoculation with a lethal dose that was previously determined in a LD50. The relative percent survival was calculated using the formula: RPS = [1 – (% mortality in vaccinated fish/% mortality in control fish] x 100%. For leukocyte analysis, tissue and peritoneal single cell suspensions (40 µm pore size nylon mesh) were centrifuged onto a 30/51% discontinuous Percoll gradient. Labelled cells were analyzed in a CytoFlex flow cytometer. Real-time PCR was performed in a QuantStudioTM 3 System (Applied Biosystems).

## Results

Challenges with *A. salmonicida* three months after vaccination resulted in a 45-54% relative percent survival for both, the oral HD and the LIPV ip. vaccines, while the RPS of the STV was 18% (Fig. 1).

After immunization, only ip. vaccines activated gene expression related to the fish immune response and increased antibody production. Higher IgM specific titters against the bacteria were obtained in the serum of fish vaccinated with the novel LIPV formulas compared to the STV which correlates with the degree of protection. In contrast, no correlation was found between the degree of protection of the oral formulas and the IgM specific titters, indicating that other immune mechanisms are also involved in fish protection against the bacteria. $IgM^+$ B cells among the cell populations in the spleen and peritoneal cavity were quantified using flow cytometry. Higher percentages of $IgM^+$ cells were found in the peritoneal exudate of the groups vaccinated ip. compared to control at 7 days post-vaccination, including LIPV and STV, that cannot explain the differences in the degree of protection between both groups. As for expression studies, strong involvement of the markers for an inflammatory response were observed at 3 and 7 days post-vaccination only in the ip. vaccinated groups including *il-1b, il-8* and *hepcidin,* and no differences were found in the expression of markers for the specific humoral and cell-mediated responses at these early time-points.

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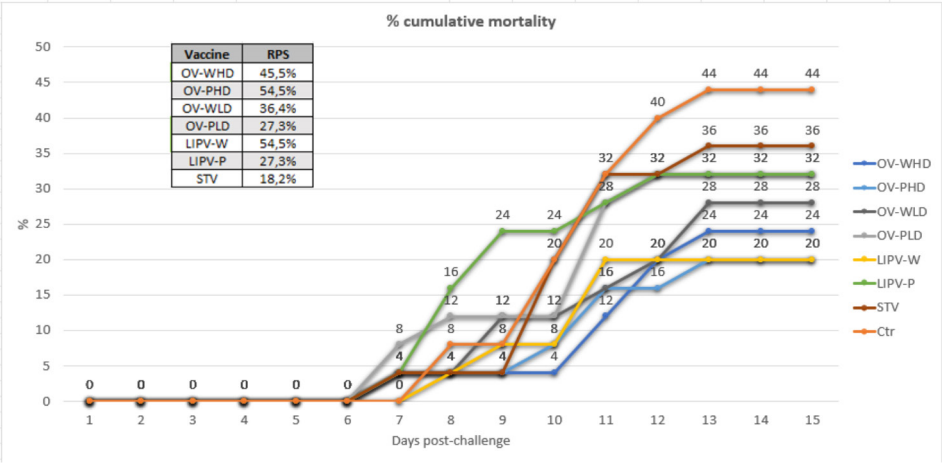


Fig. 1. Protection levels of the vaccines at 3 months post-vaccination. Cumulative mortality of *S. senegalensis* following ip. challenge with *A. salmonicida*. The RPS is indicated in the table. N = 25.

**Conclusions**

- Oral and liposome+gel-based adjuvanted vaccines confer longer-term protection than standard oil-based adjuvanted vaccines in sole against *A. salmonicida* subsp. *achromogenes*.
- Ip. vaccines induce classical systemic responses such as increment of specific antibodies in serum, recruitment of cells to the peritoneal cavity, and up-regulation of pro-inflammatory genes.
- The protective effect of the oral vaccine must rely on immune mechanisms different from those triggered by the ip. route.

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# TRANSCRIPTOMIC INSIGHTS INTO ATLANTIC SALMON GILL REGENERATION

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**Introduction**

Gill structural integrity and functionality are essential for fish survival, performance, and overall fitness in wild and aquaculture environments. Inside open sea cages, farmed Atlantic salmon are vulnerable to a variety of gill-damaging stressors, such as jellyfish, harmful algae blooms, farm management practices, and infection. Gill lesions and diseases have led to significant economic losses in Atlantic salmon aquaculture globally.

Fortunately, recent findings show that Atlantic salmon gill can regenerate following damage, restoring respiratory and ion regulatory functions (Ghanizadeh-Kazerouni et al., 2024). Building on this discovery, the present study investigates transcriptomic changes in regenerating gill filaments over 20 weeks following resection.

**Materials and Methods**

Post-smolt Atlantic salmon maintained in freshwater were lightly anaesthetised with MS-222 and subjected to gill filament resections of either 30% or 50% of filament length. Control fish underwent anaesthesia without resection. Fish from the Control and Resected groups were terminally sampled at 1, 2, 4, 8, 12, 16, and 20 weeks post-resection to collect intact and resected filaments, respectively. The 50% resected filament group was chosen for RNA sequencing as that group was expected to show more robust transcriptomic responses.

Four to seven filaments per condition and time point were processed for total RNA isolation and submitted to Canada's Michael Smith Genome Sciences Centre (Vancouver, BC) for library preparation and sequencing on an Illumina NovaSeq 6000 (PE150, targeting ≥50 million reads per library). Raw data were quality-checked and analysed using an established bioinformatics pipeline (Eslamloo et al., 2022), and differential expression analyses were performed with DESeq2 comparing Control and Resected groups at each time point, using an adjusted p-value < 0.01 and requiring expression in at least half the replicates of at least half the groups.

**Results and Discussion**

During the trial, gill filament regeneration became evident by week 8 and reached an average of 34.2% regrowth by week 20, accompanied by restored $Na^+/K^+$-ATPase activity (Ghanizadeh-Kazerouni et al., 2024). In parallel, transcriptomic differences between Resected and Control filaments diminished as healing and regeneration progressed, with 2,574 differentially expressed genes (DEGs) at week 1 post-resection, 970 DEGs at week 2, 782 DEGs at week 4, 610 DEGs at week 8, 526 DEGs at week 12, 216 DEGs at week 16, and 273 DEGs at week 20.

A hierarchical clustering analysis helped identify distinctive regulation patterns over time among the DEGs, linked to specific biological processes via Gene Ontology enrichment analyses. Inflammation-related transcripts were markedly elevated at week 1 but declined rapidly thereafter. Genes involved in cell proliferation, migration, and differentiation—including pluripotent, epithelial, and neuroepithelial cells—were also highly expressed in early stages (weeks 1 & 2), consistent with immunohistochemical observations (Ghanizadeh-Kazerouni et al., 2024). A similar temporal pattern was observed for genes related to angiogenesis, mesenchymal cell transition, cytoskeletal organisation, and cell junction assembly.

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Notably, up-regulated DEGs associated with cellular energy metabolism and organ development displayed a progressive rise in transcript levels between weeks 1 and 8—coinciding with the occurrence of blastema-like structures (Ghanizadeh-Kazerouni et al., 2024)— followed by a decline and stabilisation by week 16. These patterns suggest a phased regeneration process, beginning with rapid immune resolution and activation of progenitor cells, followed by tissue patterning and structural remodelling.

DEGs with reduced transcript levels in the Resected filaments exhibited a progressive—though often incomplete—return towards Control-like expression over time. The most robustly over-represented biological processes among these genes included ion transport (e.g. chloride, potassium, and sodium), synaptic signalling and potentiation, and lipid metabolism—processes critical in establishing functionality.

Interestingly, a distinct cluster of downregulated genes showed transcript levels rising rapidly after week 2, surpassing those in Control filaments by week 4. This cluster was enriched for fibroblast growth factor (FGF) signalling and associated processes, including glycosaminoglycan metabolism, cartilage development, extracellular matrix (ECM) components, and ECM remodelling. This pattern might reflect parallel recruitment and activity of fibroblasts and chondrocytes during structural remodelling, supporting the re-establishment of filament architecture.

In summary, our RNA sequencing results describe the transcriptomic changes underlying gill filament regeneration in Atlantic salmon as a phased process, including immune resolution, progenitor cell activation, cell proliferation, and tissue remodelling and development. Further analyses will focus on genes correlated with regeneration rates to identify molecular drivers and potential markers of successful tissue restoration.

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# ASSESSING THE EFFECTS OF TAURINE ON STRESS AND ANTIOXIDANT RESPONSES IN EUROPEAN SEABASS (*Dicentrarchus labrax*) FED LOW-FISHMEAL DIETS

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**Introduction**

Advancing sustainable aquaculture practices requires the replacement of finite marine-derived proteins in fish diets with alternative, eco-friendly sources to reduce environmental impact and reliance on limited resources. While plant-based proteins and land-animal by-products are already commonly used, their deficiency in taurine, a conditionally essential amino acid for marine fish, raises important nutritional and physiological concerns. Taurine plays a crucial role in several biological processes in fish, including growth and development during early life stages, osmoregulation, bile acid metabolism, and antioxidant defense (Aragão et al., 2022).

Throughout the production cycle, fish are exposed to various human-induced stressors, particularly during routine procedures such as grading and vaccination. These practices involve crowding, chasing, and air exposure, which can trigger acute stress responses. Such events may lead to significant physiological disruptions, including changes in endocrine function, which can negatively impact stress and antioxidant responses, potentially increasing mortality or disease susceptibility (Ciji & Akhtar, 2021)

Understanding how nutritional strategies can modulate stress responses is essential for improving fish welfare and promoting sustainable aquaculture practices. Thus, the aim of this study was to evaluate the effect of taurine supplementation in low fishmeal diets on oxidative status and stress response of European seabass following a stress challenge.

**Materials and Methods**

Three isonitrogenous, isolipidic and isoenergetic diets were formulated using practical ingredients: a control diet (**MARINE**), formulated as a premium diet rich in marine ingredients; a low fishmeal diet (**PAP**), containing only 5% fishmeal with marine ingredients replaced by animal by-products, namely poultry meal and poultry blood meal: and a diet with a formulation similar to the PAP diet but supplemented with 1% of L-taurine (**TAU**).

Triplicate groups of 23 juvenile European seabass (34.5 ± 7.4 g) were arbitrarily distributed into 100 L cylindrical tanks in a recirculating aquaculture system. Each diet was randomly assigned to three replicate tanks. Fish were fed by hand until apparent satiation, twice daily for 15 days. Tanks were supplied with filtered seawater at 20.1 ± 0.2 ºC. Dissolved oxygen, nitrogenous compounds and pH were checked and registered daily and maintained at levels within the recommended limits for the species. Fish were exposed to a natural summer photoperiod.

At the end of the feeding period, and before the stress challenge, five fish from each tank were sampled (plasma, liver, and anterior intestine) to assess the basal physiological status (**T0**). Immediately after this sampling, fish were subjected to an acute stress event (overcrowding, net chasing and one minute of air exposure) and were returned to their respective tanks. The same number of fish and biological samples that were collected at T0 were also taken at 1, 4 and 24 hours post-stress, thereafter referred to as **T1**, **T4** and **T24**, respectively.

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**Results and Discussion**

At T0, no significant differences were observed in the analyses conducted for either the stress response or antioxidant status, indicating that the replacement of fishmeal with animal by-products and the addition of taurine did not significantly affect the basal levels of these parameters. Regarding the stress response results, no significant differences were observed in glucose, cholesterol, lactate, or triglyceride levels at any time point. However, for cortisol, significant differences were detected at T1 and T24, with fish fed the TAU diet showing lower levels compared to those on the other two diets. These findings suggest that taurine supplementation at this level may exert beneficial effects by modulating cortisol release in response to stress.

As for the antioxidant status, in the liver, significant differences in catalase activity were observed at T1, with fish fed the TAU diet exhibiting significantly lower catalase activity compared to those on the other two diets. At the same time point, total glutathione levels were significantly higher in fish fed the MARINE diet than in those fed the PAP and TAU diets. Additionally, at T24, fish fed the MARINE diet showed significantly lower levels of lipid peroxidation compared to the other two groups. Regarding the anterior intestine, significant differences in catalase activity were observed only at T24, with fish fed the MARINE diet showing a significant increase in activity.

These initial findings suggest that diets rich in marine ingredients provide more effective protection against oxidative stress by reducing lipid damage and supporting both enzymatic (catalase) and non-enzymatic (total glutathione) antioxidant defenses. In contrast, the PAP and TAU diets exhibit more limited effects on antioxidant responses. However, taurine supplementation appeared to confer beneficial effects on the stress response. Therefore, while taurine supplementation may have the potential to enhance the resilience of fish fed low-fishmeal diets, further refinement of these formulations is needed to improve their ability to mitigate oxidative stress effectively.

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# ANTIMICROBIAL AND ANTIPARASITIC POTENTIAL OF ORGANOSULFUR COMPOUNDS FROM *Allium Cepa* IN SALMONID AQUACULTURE

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**Introduction**
The intensive production practices in salmonid aquaculture have led to significant health challenges, predominantly from bacterial, fungal, and parasitic infections (Tobback et al., 2009; Du et al., 2015; Wahli & Madsen, 2018; Shreves et al., 2024). Conventional use of antibiotics and antiparasitic treatments has raised global concerns regarding antimicrobial resistance and ecological sustainability (FAO/OIE/WHO, 2006). This research aims to evaluate the efficacy of two organosulfur compounds, Propyl-Propane-Thiosulfinate (PTS) and Propyl-Propane-Thiosulfonate (PTSO), extracted from *Allium cepa*, as potential alternatives for managing these infections in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*).

**Materials and Methods**
*In vitro* assays, including disk-diffusion and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC), assessed antimicrobial and antiparasitic activities of PTS, PTSO, and their combination (PTS/PTSO). Pathogens tested included *Yersinia ruckeri*, *Aeromonas salmonicida*, *Lactococcus garvieae*, *Piscirickettsia salmonis*, and *Saprolegnia parasitica*. Antiparasitic efficacy was evaluated against adult *Caligus rogercresseyi* in controlled laboratory settings. Complementary *in vivo* studies involved dietary supplementation trials in rainbow trout to determine survival after pathogen infection.

**Results**
*In vitro* results showed substantial inhibitory activity against the targeted pathogens and parasites, with PTSO demonstrating consistently greater effectiveness compared to PTS. The combination of PTS/PTSO markedly reduced parasite viability, especially in early parasite developmental stages. *In vivo* dietary supplementation with PTS/PTSO significantly enhanced survival rates and decreased disease severity in challenged rainbow trout.

**Conclusion**
The findings underscore the promising antimicrobial and antiparasitic properties of PTS and PTSO, positioning these organosulfur compounds from onion as sustainable and effective alternatives for managing health issues in salmonid aquaculture. Further research is required to optimize delivery systems and compound stability under practical farming conditions.

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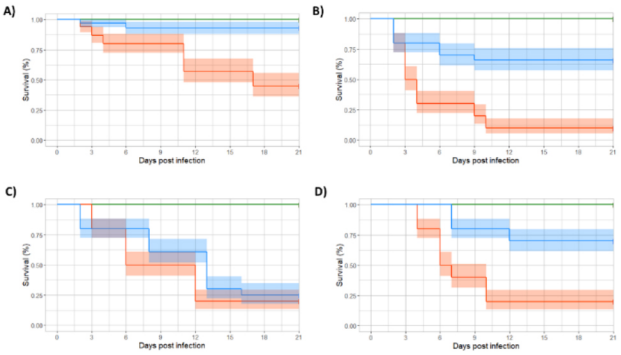


**Figure 1.** Kaplan–Meier survival curves of rainbow trout (*O. mykiss*) subjected to experimental challenges with A) *A. salmonicida*, B) *Y. ruckeri*, C) *L. garvieae* and D) *S. parasitica*. The green line represents the uninfected control group, the red line corresponds to the infected control group without treatment, and the blue line represents the infected group treated with a PTS/PTSO-enriched diet. Shaded areas indicate 95% confidence intervals.

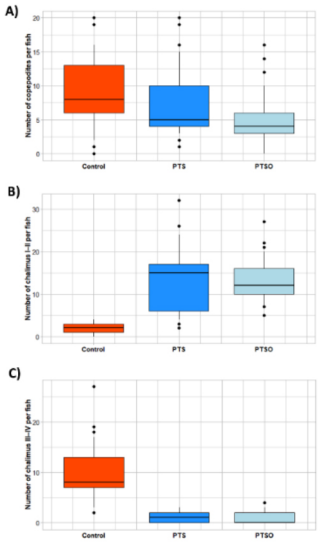


**Figure 2**. Effect of PTS- and PTSO-supplemented diets on the abundance of C. rogercresseyi at different developmental stages in Atlantic salmon. (A) Copepodite counts at 48 hours postinfestation. (B) *Chalimus* stages I–II counts at 7 days post-infestation. (C) *Chalimus* stages III–IV counts at 7 days post-infestation. Data are represented as boxplots showing the median, interquartile range, and individual outliers.

# FOOD SAFETY AND ANIMAL WELFARE IN A NEW RECLAIMED WATER-BASED AQUAPONIC SYSTEM

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## Introduction

Aquaponic production is recognized as a sustainable agriculture solution, effectively utilizing the nitrogen by-products from aquaculture as nutrients for fresh produce (Verma A.K., et al., 2023). AWARE (*Aquaponics from wastewater reclamation* GA N.101084245) aims to further this concept by establishing the first European Recirculation Aquaponics System (RAS) for using reclaimed water to grow fish (tilapia) and plants (lettuce) for human consumption. This study intends to assess the food safety of foods produced in a small-scale experimental recirculating aquaponics system that utilizes reclaimed water while ensuring animal welfare.

## Materials and Methods

**Aquaponics experimental system**: experiments were conducted in a small-scale, Recirculatory Aquaculture System (RAS) installed in Castellana Grotte coupled with a municipal wastewater plant. The experimental system comprised three different RAS modules, RAS1, 2 and 3. RAS1 operated using dechlorinated tap water (CONTROL), while RAS2 and RAS3 were supplied with reclaimed water. RAS were stocked with Nile tilapia (*Oreochromis niloticus)* and a 5 $m^2$ of crop planting surface with capacity for 90 lettuce plants (*Lactuca sativa* Batavian Red). Microbiological and chemical hazards, along with animal welfare, were monitored by collecting samples at the beginning (M0) and every three weeks at M1 and M2 between September and December 2024.

### Microbiological analysis

*Microbiological hazard analyses. Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* and intestinal enterocci were determined in tilapias and lettuces produced in the new aquaponics system accordingly with EU regulation (EC 2073/2005; EU 2020/2184; 2020/741) by following the standardized ISO methods. *Aeromonas hydrophila* and *Pseudomonas aeruginosa* were also included as non-regulated pathogenic bacteria associated to aquaponics foods (Dorick J. et al., 2024). The presence of several human enteroviruses, including Sapovirus (SaV), Norovirus (NoV GI and NoV GII), Hepatitis A (HAV), Hepatitis E (HEV) was determined by real time PCR following the ISO 15216-1; 2017. CrAssphage, and Pepper Mild Mottle Virus (PPMoV) was also assessed as indicators of fecal contamination in aquatic environments that can reflect the effectiveness of treatment systems (Gyawali, P. et al. 2019).

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*Microbiological characterization.* Total DNA, extracted from tilapia's gut and gills and lettuce's roots, in parallel with water and biofilter samples, was used for 16S rRNA gene metabarcoding aiming the characterization of the bacterial community and assessment of potential cross-contamination. In addition, the genotypes of bacterial isolates of tilapia and lettuce were compared.

***Chemical hazards analysis***: Regulated contaminants and maximum levels defined in the EU Regulation No 1881/2006 (e.g. nitrate, mycotoxins, certain metals (Pb, Cd, Hg, Sn), dioxins and PCBs, PAHs) and unregulated contaminants (CECs) were determined by means of ICP-MS, GC-HRMS and LC_HRMS.

***Animal welfare***: Animal welfare assessment was determined by combining growth performance, somatic indices, differences in inflammatory gene markers.

**Results**

***Microbiological analyses****: Regarding microbiological hazards,* no significant differences in food-borne pathogenic bacteria were observed between tilapias and lettuces produced with potable (RUN1) and reclaimed water (RUN2, RUN3). *L. monocytogenes* was only detected in the soil at M0. *Salmonella* spp. was detected by qPCR but its presence was not confirmed by the ISO method. *E. coli* and *Enterococcus* were quantified in tilapia, plants, and soil at the beginning of the experiment (M0). *Aeromonas hydrophila* and *Pseudomonas aeruginosa* were not detected. Regarding virus analysis, only NoV GI was detected in muscle al levels lower of the LoQ. Other enteric viruses including SaV, HEV or NoVGII were detected in internal organs mainly in M2. NoV GII and SaV, as well as PMMoV, were identified in the leaves of the lettuces in M2. Additionally, higher levels of PMMoV in Tilapias were observed in M2 compared to M0 and M1. Regarding *microbial characterization*, the bacterial community analysis evidenced distinct bacterial communities in tilapia and in lettuce, which were relatively stable during the experimental cycle. The hypothesis of possible cross-contamination between water, tilapia and lettuce was not confirmed based on the community analysis. However, the inspection of the genotypes of the bacterial isolates, which is more sensitive, may provide further information.

***Chemical hazards***: Initial results indicate that the levels of contaminants are well below the maximum levels defined in the EU Regulation No 1881/2006.

***Animal welfare****:* No significant differences in fish mortality were observed among the different treatments. The evaluation of growth performance revealed significant differences between RAS2 and RAS3 (ANOVA $F(2,6)=7.62$, $p=0.02$); however, these did not differ significantly from RAS 1. Similar patterns were observed for fish size. Fish in RAS 2 showed increased length, although the differences were not statistically significant (ANOVA $F(2,6) = 4.65$, $p = 0.06$). The hepato-somatic index indicated reduced liver weight in RAS1, but this difference was not significant ($F(2,6)=2.92$, $p = 0.13$). In contrast, the spleen-somatic index showed significant differences among treatments ($F(2,6)=12.5$, $p=0.007$). Specifically, RAS1 differed significantly from both the control ($p < 0.01$) and RAS 2 ($p = 0.01$).

**Conclusions**: the first conclusion is that microbiological risk analysis of tilapia and lettuce produced in the aquaponic system designed at AWARE using reclaimed water showed that these foods are safe. Additionally, there was no evidence of microbiological cross-contamination between water, tilapia and lettuce. Regarding chemical hazards, analysis indicate that the levels of contaminants are below the maximum levels defined in the EU-regulation. Finally, fish mortality remained unaffected across treatments, although physiological responses suggest differing resource allocation strategies.

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# ELEVATED VITAMIN LEVELS ENHANCE WOUND HEALING EFFICIENCY IN RAINBOW TROUT

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## Introduction

Lesions in the skin are a major welfare problem for the fish and a big cause of economic losses for the salmon industry (Takle et al. 2015). Skin lesions, abrasions, and ulcers are the main entrance for pathogens. In these situations, skin wounds must be promptly treated in order to avoid negative impacts on fish (Ceballos et al. 2017 and Costa et al. 2018). The wound healing process is dependent on factors such as temperature, wound dimensions, stress, and importantly nutrition. However, few studies have investigated nutritional interventions that could potentially improve the regeneration of fish skin (Sveen et al 2019). The aim of this study was to test the effects of vitamin inclusion level on wound healing. To this end a skin wound healing model was created in salmonids to evaluate the molecular and biochemical process involved in the different phases of regeneration in rainbow trout.

## Material and Method

The trial was performed at the Research Centre for Animal Nutrition and Health (DSM Nutritional Products France). Eighty rainbow trout (*Oncorhynchus mykiss; initial body weight 63g*) were randomly allocated to two different experimental groups. The fish were fed 84 days with basal diet mimicking a Norwegian commercial recipe (control) or an Optimum Vitamin Nutrition (OVN) diet (Liu et al 2022) that differed from the control in higher vitamin level inclusions. Each treatment was given to two replicate groups of 20 fish Animals from the 2 diets were wounded after 28 days of feeding with the experimental diets corresponding to day 0 of the experiment. The fish were wounded with a punch biopsy tool, transversing the full thickness of the skin barrier on the left dorsal flank of the fish. The principal advantage of this model is that a deep wound involves both dermal and epidermal repair processes. The fish continued to be fed with the experimental feed until the end of the trial. Growth parameters, blood biochemistry, wound size, and the expression of 12 genes representative of the different phases of the wound healing process were monitored at day 0, 1, 3, 7, 14, 21, 28, 35, 49 and 56 days post wounded (dpw).

## Results

As expected, strong modulations over time were observed for all the blood parameters, except for phosphorus ($p=0.0870$) and sodium ($p=0.1035$). An increase in the size of the wounds on 1dpw, 3dpw, and 7dpw was observed. The return of the initial size of the wounds occurred for the two treatments between 7dpw and 14dpw. With high levels of vitamins diet a complete closure of all the wounds occurred 7 days before those of fish fed with control diet. After normalization of the size of the wounds to the 0dpw size for each fish, statistical differences were observed between the two treatments at 1dpw ($p=0.0028$), 3dpw ($p=0.0014$) and, 7dpw ($p=0.0072$). From 14dpw to the end of the trial, no differences were observed between the treatments ($p>0.05$) (Figure 1).

We were able to identify the progression of wound healing at the transcriptomic level. The re-epithelialization genes followed an expected pattern, but maximum expression was delayed. For MMP13 we observed maximum expression at 14dpw for diet J and 7dpw for control diet. Maximum expression of chemokine 4 ligands was observed at 3dpw for the two diets. This may reflect the fact that immune cells are less necessary at the very beginning of the wound healing process in our model. The inflammation step was reduced in this trial. The expression of serum amyloid A appeared early, at 3dpw for both diets. In addition, IL-1B gene expression levels were flat with many variations. Granulation tissue genes are also affected in our model. Instead of a continuously decreasing expression expected, we observed a maximum of expression at 14 days with the two diets. This could indicate a lack of activation of the gene at the very beginning of the wound. The genes involved in tissue remodeling (Col1a1a and Hyaluronisase-2-precursor) showed an expected profile, similar between the two diets.

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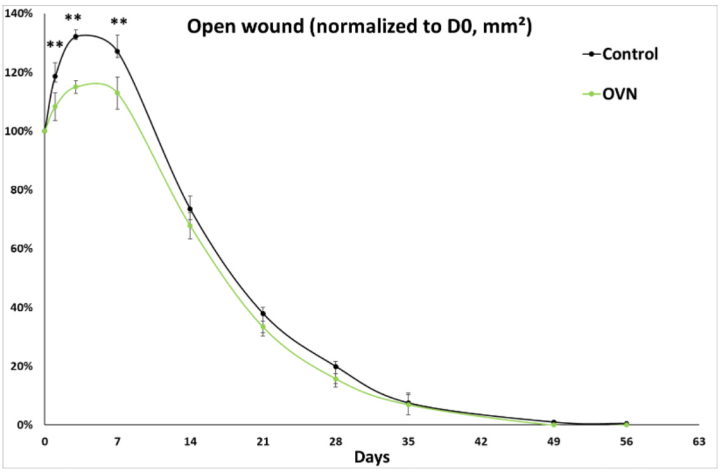


Figure 1: Size of the open wound over the time. * corresponds to significant differences between the treatments (**: $p<0.01$). Abbreviation: OVN, Optimum Vitamin Nutrition.

The genes involved throughout the wound healing process (Cytochrome P450 4F3 and Epigen precursor) showed also an expected expression profile, reflecting the success of the wound healing process. Interestingly, CD4 gene expressions were limited to T0 and 3dwp. The level of CD4 expression was lower with control diet K than with OVN diet.

**Conclusion**

The model presented was able to assess the difference between the inclusion levels of vitamins. In this trial based, we were able to observe differences macroscopically but also at transcriptomic level. And, it is clear that vitamins play a key in supporting wound healing. Then, the reduction of vitamin inclusion levels in Salmonid diets should be done carefully.

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# STUNNING AND SLAUGHTER METHODS IN GILTHEAD SEABREAM: ANIMAL WELFARE AND MUSCLE QUALITY ASSESSMENT

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**Introduction**

Global aquaculture production has surpassed capture fisheries, reaching 111 million tonnes in 2022, with projections to continue growing. This expansion raises concerns over fish welfare, particularly in relation to humane stunning and slaughter methods. The EU has established directives emphasising the minimization of suffering, especially during slaughter. While methods like electrical and mechanical stunning are deemed humane, practices such as ice asphyxiation and $CO_2$ exposure are considered inhumane. Despite increased awareness, many fish, including gilthead seabream, are still slaughtered without stunning, resulting in significant stress and muscle deterioration. This study investigates the effects of different stunning and slaughter techniques (electrical stunning, anaesthesia, and no stunning) followed by either ice-slurry or *ikejime* on the welfare and meat quality of gilthead seabream.

**Materials and Methods**

Conducted at the Estação Piloto de Piscicultura de Olhão (EPPO) in Portugal, this study involved 32 adult gilthead seabream. Fish were housed in three controlled outdoor tanks and acclimatized for 7 days before bio-loggers were surgically implanted to monitor heart rate and internal temperature. After a recovery period, the fish were subjected to various stunning and slaughter treatments: electrical stunning, anaesthesia, or no stunning, followed by either ice-slurry or *ikejime*. Physiological and behavioural data, including heart rate, blood samples, and muscle quality parameters, were collected. Data were analysed using linear mixed models (LMM) for heart rate, internal temperature and blood parameters, one-way ANOVA for muscle quality, and PCA for muscle quality and plasma datasets.

**Results**

We found notable differences in fish behaviour, heart rate, physiological responses, and muscle quality based on the stunning/slaughter method employed. Electrical stunning led to an immediate loss of visual consciousness, except for one fish that retained its vestibulo-ocular reflex (VOR). Fish slaughter with ice-slurry showed delayed loss of movement and more stress indicators, as well as significantly lower heart rates compared to those slaughtered with *ikejime*. Cortisol levels were highest in fish slaughtered by ice-slurry without prior stunning, indicating greater stress. Glucose levels were elevated in most treatments, with the highest increase seen in fish subjected to anaesthesia followed by *ikejime*. Other physiological markers, such as lactate, calcium, and potassium levels, varied significantly with the treatment. Electrical stunning followed by *ikejime* resulted in higher lactate and potassium levels. Muscle quality analyses showed that fish slaughtered only with *ikejime* or previously stunned with anaesthesia had softer, less chewy, and more water-retentive muscle than fish slaughtered with ice-slurry or electrically stunned. These two later groups also resulted in greater water and fat loss than the fish anaesthetised before slaughter.

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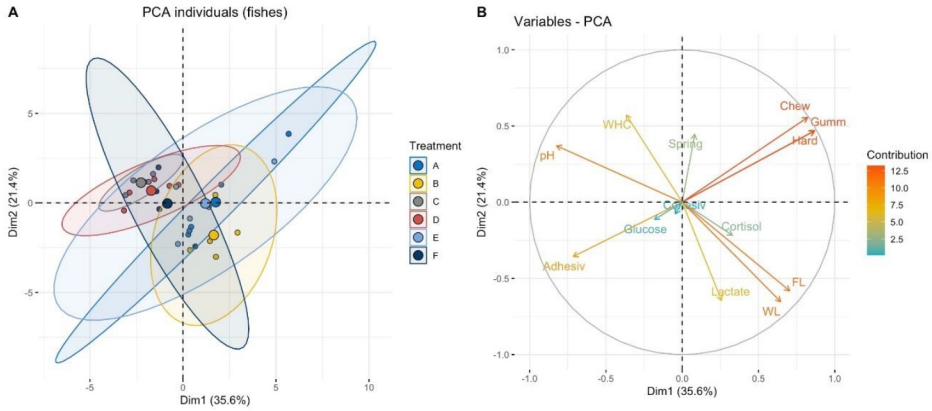


*Figure 1. PCA plots of individuals (left) and variables (right) of muscle considering the muscle quality data. PCA plot of individuals: the 95% confidence ellipses group fishes according to treatment. PCA plot of variables: The larger the value of the contribution, the more the variable contributes to the component. Treatments are: A. e- stunning + ice-slurry, B. e-stunning + ikejime, C. no stunning + ice-slurry, D. no stunning + ikejime, E. anaesthesia + ice-slurry, F. anaesthesia + ikejime.*

## Discussion

This study demonstrates that different slaughter techniques significantly impact both the physiological stress responses and muscle quality of gilthead seabream. Fish that were not stunned experienced prolonged consciousness and stress, particularly when slaughtered in ice-slurry, where they remained conscious for up to 18 minutes. Although anaesthesia and electrical stunning resulted in some unconsciousness, fish still showed stress responses during slaughter with ice-slurry, suggesting that these methods may not always be fully effective when in combination with ice-slurry. Ice-slurry induced bradycardia and higher cortisol levels, with unstunned fish exhibiting a faster temperature drop and greater stress. Muscle quality was best preserved in fish stunned with anaesthesia before slaughter, while electrical stunning led to lower pH and undesirable texture. In conclusion, while anaesthesia and *ikejime* produced the best outcomes for fish welfare and meat quality, electrical stunning requires further validation, and the development of automated *ikejime* could offer a humane and effective alternative for the industry.

# A MULTIBIOTIC COMPOUND FROM MACROALGAE IN PLANT-BASED DIETS MODULATES GUT INTEGRITY AND PUTATIVE PEPTIDE TRANSPORT IN *Sparus aurata*

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**Introduction**

Several studies have shown that both microalgae and macroalgae offer significant nutritional benefits by helping prevent diseases and infections, due to their high content of vitamins, minerals, and essential fatty acids (Idenyi *et al*., 2022; Chandrasekar *et al*., 2024). These organisms have gained attention as promising natural sources for the development of nutraceutical products. One of the current challenges in aquaculture is determining whether such nutraceuticals can improve the performance of feed formulations with a high proportion of plant-based protein, particularly in commercially important species (Caderno *et al*., 2025). If successful, this approach could make aquaculture a much more environmentally sustainable economic activity. This study aims to evaluate the effects of Prebiodo II supplementation on intestinal structure and function in the context of a low-fishmeal diet, by analysing the expression of key genes in the anterior and mid-intestine of gilthead seabream (*Sparus aurata*).

**Materials and methods**

A total of 180 juvenile gilthead seabream (initial body mass: 54.58 ± 0.05 g) were randomly distributed into nine 300-L tanks (n = 20 fish per tank). Three experimental groups were established in triplicate and fed as follows: (i) a control diet containing a medium fishmeal level (12%); or the same basal formulation supplemented with either (ii) 1% or (iii) 3.5% of the nutraceutical compound Prebiodo II. Prebiodo II is a natural symbiotic nutraceutical based on macroalgae hydrolysates, alginate, and enzymatic-bacterial extracts, produced by Biotechnology Biopolym S.A. (Granada, Spain). Fish were fed *ad libitum*, with two meals per day, over a 91-day experimental period. The expression of several genes related to intestinal integrity, peptide transport, and immune response was examined in both the anterior and mid-intestinal segments.

**Results**

Similar gene expression patterns were observed across intestinal regions for the genes studied, which are involved in maintaining intestinal integrity, peptide transport, and immune function. In the anterior intestine (AI), gene expression remained stable among experimental groups, suggesting that this region maintains homeostasis despite dietary changes and supplementation with Prebiodo II.

In contrast, the mid intestine (MI) exhibited a significant downregulation of key genes (*igm, tlr9, oclb, oclc, cld4, cld7b, alpi, slc15a1, slc15a2,* and *slc15a4*) in the B1% group, whereas expression levels in the Control and B3.5% groups remained comparable (Fig. 1). This coordinated reduction may reflect a compromised intestinal barrier, impaired digestive and absorptive capacity, and weakened mucosal immunity. Specifically, the decreased expression of tight junction proteins (occludins and claudins) suggests increased intestinal permeability, while the downregulation of peptide transporters and immune markers may indicate deficits in nutrient uptake and local immune defense.

These findings suggest that Prebiodo II supplementation at a 1% inclusion rate negatively affects intestinal health in the mid-intestine, despite the absence of detrimental effects on growth performance and somatic indices at any tested level. Further investigation is warranted to elucidate the molecular mechanisms underlying this apparent dose-dependent response and to determine its potential long-term implications.

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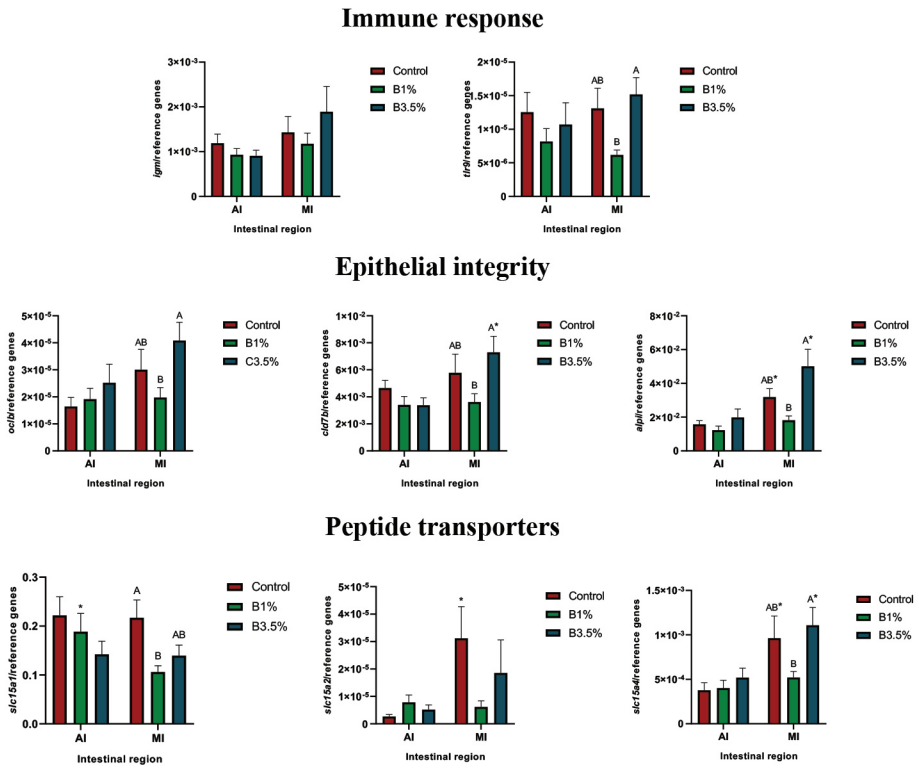


**Fig. 1.** Gene expression levels of *igm*, *tlr9*, *oclb*, *cld7b*, *alpi*, *slc15a1*, *slc15a2*, and *slc15a4* in the anterior (AI) and mid (MI) intestine of *Sparus aurata* fed different experimental diets (Control, B1%, and B3.5%). Data are expressed as mean ± SEM (n = 12 fish per experimental group). Different superscript letters indicate statistically significant differences among dietary treatments, while asterisks denote significant differences between intestinal regions within the same dietary treatment, as determined by two-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

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## Acknowledgments

This work was supported by Project OTRI-2021/134, funded by Biotechnology Biopolym S.A. (Granada, Spain). Anyell Caderno has been supported by a Ph.D. fellowship from the Regional Government of Andalusia (2021, PREDOC_02015).

# A DETERMINISTIC COMPARTMENTAL MODEL FOR FLAVOBACTERIOSIS CONTROL IN RAINBOW TROUT FARMING: FROM EPIDEMIOLOGICAL INSIGHTS TO MANAGEMENT AND GENETIC SELECTION STRATEGIES

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**Statement of the Problem**

Flavobacteriosis, caused by *Flavobacterium psychrophilum*, is a major threat to rainbow trout (*Oncorhynchus mykiss*) aquaculture, with outbreaks causing up to 70% mortality [1]. Current control strategies are limited, and understanding the epidemiological drivers—such as pathogen excretion, transmission efficiency, and environmental factors—is crucial for effective intervention. While existing studies focus on resistance through challenge experiments, few have developed comprehensive epidemiological models. Agent-based approaches, like that of Brenden et al.[2], provide simulations but lack analytical insights. To address this, we propose a deterministic compartmental model that enables both numerical and analytical examination of flavobacteriosis dynamics. This model offers practical guidance for managing aquaculture parameters during outbreaks and identifies key targets for genetic selection, advancing disease control strategies.

**Modeling Approach**

Our model follows a SIR-type framework [3]. It represents fish populations through compartmental states: susceptible ($S$), exposed but non-excreting ($E$), infected ($I$) and excreting (at rate $\beta_{I\to P}$), recovered and immune ($R$), and dead ($D$) but still excreting (at rate $\beta_{D\to P}$). The pathogen concentration in the system ($P$) is reduced by water outflow at rate $\gamma$. Epidemiological parameters $\beta$ govern transitions between states, while the mortality removal frequency and the water exchange $\gamma$ are aquaculture-specific factors. The full system is formalized in Equation (1).

$$\dot{S} = -\beta_{S\to E}PS \qquad \dot{R} = \beta_{I\to R}I$$
$$\dot{E} = \beta_{S\to E}PS - \beta_{E\to I}E \qquad \dot{D} = \beta_{I\to D}I - \mu D$$
$$\dot{I} = \beta_{E\to I}E - (\beta_{I\to R} + \beta_{I\to D})I \qquad \dot{P} = \beta_{I\to P}I + \beta_{D\to P}D - \gamma P$$

**Epidemic Intensity and Control**

We analytically demonstrate that epidemics always extinguish, with a subset of fish remaining uninfected. We pinpoint both farm management strategies -- such as increasing the water outflow $\gamma$ -- and critical epidemiological traits -- such as recovery rate ($\beta_{I\to R}$) and pathogen excretion rate ($\beta_{I\to P}$) -- as targets for genetic selection, aiming to optimize disease resilience in farmed populations. However, the mortality rate ($\beta_{I\to D}$) requires careful consideration: it should be maximized if $\frac{\beta_{I\to P}}{\beta_{I\to R}} > \frac{\beta_{D\to P}}{\mu}$ (prioritizing rapid removal of infected fish) or minimized otherwise (reducing overall mortality). We introduce an epidemic intensity indicator, Q=E+I+a D+ bC, where a>0 and b>0 are positive scalars dependent on model parameters. Q equals zero if and only if the epidemic is extinct. Our analysis reveals that Q exhibits only two possible behaviors: it either **rises and then declines to zero** (indicating an epidemic that takes hold before fading) or **declines immediately to zero** (indicating an epidemic that fails to establish itself). Through analytical study, we derive a critical threshold for epidemic growth: the epidemic spreads if and only if$\zeta = \frac{\gamma\mu}{\beta_{S\to E}} \frac{\beta_{I\to R}+\beta_{I\to D}}{\beta_{D\to C}\beta_{I\to D}+\mu\beta_{I\to C}} < 1$This result provides a clear target for intervention: parameters must be adjusted until $\zeta > 1$ to contain the epidemic.

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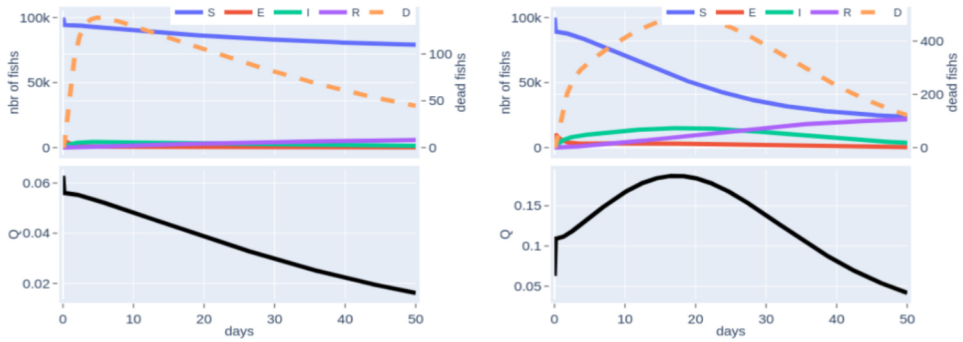


(a) $\zeta > 1$ (b) $\zeta < 1$

*Fig 1.* For each simulation, the upper panel shows the proportion of fish in each compartment (susceptible, exposed, infected, recovered, and dead) within the aquaculture system, while the lower panel depicts the epidemic intensity indicator Q. Panel **(a)** corresponds to $\zeta > 1$ , where Q declines immediately, indicating an epidemic that fails to establish. Panel **(b)** corresponds to $\zeta < 1$ , where Q initially increases before decaying to zero, reflecting an epidemic that takes hold before subsiding.

**Model Calibration and Simulation**

Calibrating this model is challenging due to the lack of comprehensive monitoring data from aquaculture systems. While existing infection experiments in the literature only allow analysis of specific epidemiological fluxes, we combined multiple datasets to calibrate the model and generate realistic simulations. Figure 1 illustrates two scenarios, corresponding to (epidemic growth) and (epidemic extinction).

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# "SEA BUGS" – MARINE GAMMARID AMPHIPODS AS A SOURCE OF *N*-3 LONG-CHAIN POLYUNSATURATED FATTY ACIDS

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**Introduction**

Marine farmed fish are praised for their nutritional value for human consumption, but their *n*-3 long-chain (≥C20) polyunsaturated fatty acids content strongly depends on their diet. For decades, fishmeal and fish oil have been used to formulate aquafeeds, a practice that is increasingly questioned, as it supplies *n*-3 LC-PUFA through fish for human consumption but also depletes wild fish resources. To tackle the generalised shortage of *n*-3 LC-PUFA rich marine ingredients, the industry started targeting plant-based ingredients. However, these are richer in *n*-6 fatty acids and lack *n*-3 LC-PUFA. These caveats negatively affect fish health, performance, and nutritional value.

The quest for the diversification of sources able to deliver suitable levels of *n*-3 LC-PUFA has focused on krill and single-cell organisms (e.g., microalgae and protists). However, most of such alternatives are still too costly for mainstream uses. Bio-engineered transgenic oilseed crops have also been put forward as an option, but their fatty acid profiles often feature an imbalanced *n*-3/*n*-6 ratio, and several countries still restrict the use of genetically modified organisms. Insects have sparked a growing interest in the industry, being considered as a promising ingredient for aquafeeds formulation, with emphasis on black soldier fly (*Hermetia illucens*). However, insects only display interesting levels of *n*-3 LC-PUFA if these are supplied through their diet, as they bioaccumulate these biomolecules, rather than biosynthesizing them *de novo*.

Marine gammarid amphipods emerge as a promising source of *n*-3 LC-PUFA, despite remaining largely overlooked. These highly diverse groups of "sea bugs", are in fact shrimp-like peracarid crustaceans (with a few mm size) that are found in multiple aquatic environments. Their trophic plasticity makes them promising candidates for circular bioeconomy approaches, aiming to add value to terrestrial food-processing by-products by upgrading them into a *n*-3 LC-PUFA rich biomass.

***n*-3 LC-PUFA profile of marine gammarids**

The ability of marine gammarids to produce *n*-3 LC-PUFA has been reasoned from feeding experiments studying the impact of different diets on the fatty acids of their body composition. While gammarids possess elongases that play a role in *n*-3 LC-PUFA biosynthesis, the full mechanism of eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) synthesis is yet to be fully understood on these organisms, as they appear to lack desaturases; these enzymes are needed for *de novo* synthesis of these fatty acids through known pathways. It has been hypothesized that this ability to synthetize EPA and DHA may result from alternative mechanisms, such as contributions from gammarids' microbiota. These "sea bugs", namely *Gammarus locusta* (Figure 1), have been shown to be able to perform trophic upgrade, as they can convert lower-value feeding substrates (e.g., seaweeds, land vegetables…) into higher-value nutrients, which ultimately improves their nutritional value as a dietary ingredient/supplement. By using diets from non-marine sources, namely agricultural by-products, to farm marine gammarid amphipods, one can support circular bioeconomy principles and enhance resource efficiency, while simultaneously reducing the needs on more traditional ingredients.

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**Conclusion**

Marine gammarid amphipods can convert both lower-value marine and non-marine feeding substrates into high-value *n*-3 LC-PUFA through trophic upgrade. With farmed amphipod gammarid offspring reaching sexual maturity at only six weeks post-hatching it may be possible to address nutritional programming and selective breeding practices, allowing to develop lineages that may even outperform wild conspecifics in terms of their *n*-3 LC-PUFA composition. Overall, this approach will strongly contribute to merge green and blue food ecosystems, contributing towards a more sustainable aquaculture.

**Acknowledgements**

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# MICROBIAL INFLUENCE ON BLACK SPOT DEFECT IN FARMED SHRIMP: THE ROLE OF BIOFLOC SYSTEMS IN MITIGATION

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## Introduction

Shrimps are one among the most traded species around the world. In the world, (*L. vannamei*) is the most desired species for cultivation owing to several advantages over other species (FAO, 2014). As in the case of any food product, spoilage in shrimps is a major concern for shrimp farmers and shrimp processing industry. The shrimp are a highly perishable product due to their rapid microbiological and biochemical spoilage post-harvest. Black spot (melanosis) is a common post-harvest defect in farmed shrimp, characterized by dark pigmentation on the shell due to enzymatic oxidation. In biofloc production systems, which enhance water quality and shrimp immunity through microbial floc, the occurrence of black spot may be influenced by factors such as water composition, stress during harvest, and microbial dynamics. While biofloc offer improved health and growth performance, their impact on post-harvest quality, including melanosis susceptibility, remains a critical area for optimization in commercial shrimp farming. The primary objective of this study is to evaluate the impact of microbial dynamics on the development of black spot disease in farmed shrimp during ice storage, while comparing different slaughter methods and investigating the potential of biofloc systems to mitigate the disease over time.

## Material and Methods

Four slaughter methods were evaluated: cold thermal shock (TS) using refrigerated seawater (-2ºC) (TREATMENT-2 -Control-) (TS was implemented in all slaughter methods); 5 min immersion in refrigerated seawater (0±2.5 ºC) with 50 ppm bleach followed by TS (TREATMENT-1); clarification (3–4 h) before 50 ppm bleach (TREATMENT-3) and TS; and TS followed by 2 min UV exposure in each side of the shrimp (TREATMENT 4). A trained sensory assessor's panel evaluated the melanosis progression, over 7 days on interval of 48 h, using the procedure "Melanosis Score Sheet" described in Montero et al (2001). Additionally, bacterial specific study (Specific Spoilage Organism -SSO-, Total Viable Count -TVC-, Pseudomonas -PSE-, Enterobacteria -ET- and Psychotropics -PST-) was carried out, together with a physicochemical parameter (non-protein nitrogen-TVB-N). The data collected were analysed using XLSTAT® statistical software, performing descriptive analysis, panel analysis, ANOVA and Fisher's post hoc tests. The results were presented using a Principal Component Analysis (PCA) as a general test.

## Results and discussion

The PCA analysis (Fig.1) of fresh shrimp stored on ice reveals critical insights into spoilage progression and the effectiveness of four different treatments. Microbiological indicators such as Specific Spoilage Organisms (SSO), Pseudomonas (PSE), and Total Viable Count (TVC) are closely grouped on the left side of the plot, correlating with early spoilage and associating with samples from DAY-0 and DAY-3. These early-stage samples also align with TREATMENT-1 and TREATMENT-2, suggesting these treatments are effective at preserving microbial quality in the initial storage period. In contrast, spoilage parameters like Enterobacter (ET), Psychrotrophics (PSY), Total Volatile Basic Nitrogen (TVBN), and black spot development point toward the right and lower quadrants of the plot, indicating their relevance in advanced spoilage. DAY-9 and especially DAY-12 are positioned along these spoilage vectors, highlighting a significant decline in quality over time. Notably, TREATMENT-3 clusters near DAY-12 and the black spot vector, indicating it was the least effective in controlling spoilage and melanosis. TREATMENT-4 shows moderate performance, with some overlap toward spoilage indicators. Overall, this analysis shows that TREATMENTS 1 and 2 provide the best preservation of shrimp quality, particularly within the first nine days of storage, while TREATMENT-3 fails to mitigate spoilage effectively.

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**Conclusions**

The study clearly demonstrates the critical influence of slaughter methods on the microbiological quality and shelf-life stability of ice-stored shrimp, from biofloc production system with melanosis (black spot) emerging as a key quality-degrading factor impacting commercial value. Among the evaluated approaches, TREATMENT-1—involving immersion in refrigerated seawater (0 ± 2.5 ºC) with 50 ppm sodium hypochlorite followed by cold thermal shock (TS)—proved the most effective, showing strong correlations with suppressed microbial loads (TVC, SSO, PSY), lower TVBN levels, and significantly delayed black spot development, particularly up to DAY-9. TREATMENT-2 (TS alone, used as control) also maintained acceptable microbial quality but was slightly less effective in controlling melanosis, which can negatively affect consumer acceptance and marketability. In contrast, TREATMENT-3 (clarification followed by bleach and TS) resulted in elevated spoilage indicators and pronounced black spot formation by DAY-12, rendering it commercially unviable. TREATMENT-4 (TS followed by UV exposure) showed intermediate performance, with no substantial advantage over the control. From a commercial perspective, TREATMENT-1 is strongly recommended for ice-storage operations targeting extended shelf life and high sensory quality, particularly for export markets where appearance is critical. It is further recommended that shrimp be marketed or processed by DAY-6 to DAY-9, and that regular monitoring of melanosis and microbial indices be implemented to ensure product integrity, reduce losses, and meet regulatory and customer expectations.

**Acknowledgments**

This work was supported by I+D+i Research Project: “Pursuing the zero waste in Biofloc Technology to produce a sustainable and biosafe aquaculture product (ZeroFloc)” (PID2023-149570OB-I00). S.Ferrando-Juan has a predoctoral Research Personnel Training Grant (PRE2021-098367); supported by MCIN/ AEI/10.13039/501100011033/. J. Gómez-Aguilera contract was supported by European Union Next Generation-Plan of Conselleria d’innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434).

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# FGF21 AS AN ENDOCRINE MODULATOR OF METABOLISM IN RAINBOW TROUT: FROM MOLECULAR CHARACTERISATION TO PHYSIOLOGICAL RESPONSE

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**Introduction**

Fibroblast growth factor 21 (FGF21) belongs to the endocrine FGF subfamily and is widely recognised in mammals as a metabolic regulator crucial for energy balance. It is mainly expressed in hepatic tissues across most species, where its expression is increased under fasting conditions. Among other functions, FGF21 promotes gluconeogenesis, ketogenesis and fatty acid oxidation (1). Furthermore, it has been observed that diets rich in carbohydrates and low in protein increase FGF21 levels, promoting protein intake and influencing food preferences. These responses are mediated centrally by FGF21, which acts as a key hormone signalling protein restriction in the brain, particularly by increasing the reward value of protein-containing foods through dopamine-related neural circuits (2)cost, and nutritional value can vary markedly. Animals have thus developed neural mechanisms that enable the detection of nutrient restriction, and these mechanisms engage adaptive physiological and behavioral responses that limit or reverse this nutrient restriction. This review focuses specifically on dietary protein as an essential and independently defended nutrient. Adequate protein intake is required for life, and ample evidence exists to support an active defense of protein that involves behavioral changes in food intake, food preference, and food motivation, likely mediated by neural changes that increase the reward value of protein foods. Available evidence also suggests that the circulating hormone fibroblast growth factor 21 (FGF21.

This information offers valuable insights that can be extrapolated to improve aquaculture feeding research, emphasising the importance of understanding and modulating the nutritional and sensory cues that drive fish feeding behaviour. Specifically, understanding the role of Fgf21 and reward pathways in regulating protein-specific appetite could lead to the development of optimized feed formulations that align with the animals' natural adaptive mechanisms for detecting and responding to protein deficiency. However, very little information is available on Fgf21 in fish. Only two studies address the metabolic role of this protein and its relation with feed intake regulation: one in zebrafish (*Danio rerio*) (3), which found that Fgf21 plays an orexigenic role and is modulated by feeding state, exerting a significant influence on glucose and lipid metabolism; and the other in Asian seabass (*Lates calcarifer*) (4)whereas potential functions of this gene in teleosts are still unknown. We identified the Fgf21 gene and studied its functions in Asian seabass (Lates calcarifer, which revealed several differences compared to the mammalian model, including a different expression pattern, fasting-induced downregulation and an anorexigenic role of Fgf21. These interspecies differences in fish, as well as differences with mammals, led us to conduct the present experiment. Using rainbow trout (*Oncorhynchus mykiss*) as a model of high economic value in aquaculture, we outline a study to characterise the metabolic role of Fgf21 and its effect on feed intake, from molecular characterisation to physiological response.

**Materials and methods**

Juvenile rainbow trout were acclimated to standard conditions: temperature (15ºC ± 1ºC), photoperiod (12 h light:12 h dark), and feeding (once daily at 10:00 h with commercial dry pellets, hand-fed until apparent satiety). Fish were maintained in 100 L tanks with aerated, dechlorinated tap water in an open recirculating system. For the tissue distribution analysis, 6 randomly selected fish were fasted for 48 h, sacrificed, and various tissues were collected. Total RNA was then extracted to quantify relative *fgf21* mRNA levels by RT-qPCR. Additionally, to examine the daily profile of *fgf21* mRNA abundance in the hypothalamus, a second group of 42 animals maintained under standard conditions was sampled every 4 hours throughout the light/dark cycle (n = 6 per sampling point), starting at ZT0 (lights on), and continuing at ZT4, ZT8, ZT12 (lights off), ZT16, ZT20, and ZT24 of the following day. Primers for *fgf21* were previously designed using Primer designing tool from NCBI, and their specificity and effectiveness were validated. Daily rhythms of gene expression were assessed by fitting a single cosine function to the relative mRNA abundance values across the seven time points using the Cosinor.Online application.

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In a second experiment, fish were individually weighted and distributed in 3 tanks (n = 13 / tank). During the acclimatation period, fish were fed twice daily, and basal levels of feed intake were recorded. On the day of the experiment, animals were intraperitoneal (IP) administered with one of the three treatments: saline solution alone (control) or containing either 100 ng/g or 200 ng/g of recombinant Fgf21. Feed intake was recorded at 2, 8, 26, 32, 50, 56 h post-administration. The experiment was repeated four times, replacing the animals in each replicate. Finally, a fifth replicate was performed to collect tissue samples at 2 and 8 h post-administration. Fish were sacrificed and samples of liver, adipose tissue, and hypothalamus were collected for qPCR analysis of various genes related to feed intake regulation and metabolism. For statistical analysis, a two-way ANOVA was conducted using RStudio 2024.12.1, considering treatment (control, 100 ng/g, and 200 ng/g Fgf21) and time (2 h and 8 h) as main factors. When significant effects or interactions were detected ($p < 0.05$), post-hoc Tukey's multiple comparisons tests were performed.

**Results and discussion**

Analysis of gene encoding Fgf21 distribution in rainbow trout revealed a generally higher expression in central regions compared to peripheral tissues. Notably, unlike in mammals, no expression was detected in the liver of rainbow trout, a pattern also reported in Asian seabass (4)whereas potential functions of this gene in teleosts are still unknown. We identified the Fgf21 gene and studied its functions in Asian seabass (Lates calcarifer, supporting the idea that Fgf21 functions differ between fish and mammals. Furthermore, hypothalamic mRNA abundance of *fgf21* displayed clear daily oscillations, peaking around the beginning of the dark phase (ZT12). This pattern suggests that Fgf21 may be involved in the circadian regulation of metabolism or feeding behaviour.

The IP administration of recombinant Fgf21 in rainbow trout resulted in a reduction of feed intake, which was more pronounced at 2, 8, and 26 h post-administration of the high dose. This anorexigenic response is consistent with findings previously reported in Asian seabass (4)whereas potential functions of this gene in teleosts are still unknown. We identified the Fgf21 gene and studied its functions in Asian seabass (Lates calcarifer. In the hypothalamus, Fgf21 increased *cartpt* and *pomca1* mRNA levels, particularly at 8 h post-injection, suggesting activation of anorexigenic pathways consistent with the observed reduction in feed intake. In parallel, *ampkα2* was also upregulated at 8 h with the high dose, indicating activation of central energy sensing mechanisms. Furthermore, there was a non-significant trend towards increased levels of *fgf21* and its receptor *fgfr1a2*, which suggests a potential enhancement in central sensitivity to Fgf21. In the liver, Fgf21 induced a lipid catabolism shift, upregulating *pparα* and *cd36* while downregulating *glut2* and *fbp*. Additionally, *ampkα1* increased and *mtor* decreased, reflecting activation of energy-saving mechanisms consistent with the central response. In adipose tissue, Fgf21 promoted a time-dependent regulation of lipid metabolism, with early activation of *hadh* (2 h) and later induction of *cpt1b* (8 h). Lipogenesis was suppressed by the high dose of Fgf21 via downregulation of *srebp1c* and *lxrα*, while *pfk* and *fbp* also decreased, indicating reduced glucose use and a switch to lipids as preferred energy source.

Overall, these findings suggest that Fgf21 plays a key role in the regulation of energy balance, feeding behaviour, and lipid metabolism in rainbow trout. The distinct tissue distribution, daily oscillations in hypothalamic expression, and the metabolic and anorexigenic responses to exogenous Fgf21 administration highlight functional differences between fish and mammals and point to a species-specific role of Fgf21 in coordinating central and peripheral energy homeostasis.

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# BIOCONCENTRATION AND DEPURATION OF ORGANIC POLLUTANTS IN EUROPEAN EELS AND THE INFLUENCE OF MICROPLASTICS

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## Introduction

Organic pollutants (OPs) of anthropogenic origin are widespread in surface water globally including pharmaceuticals and personal care products (PPCPs), per- and polyfluoroalkl substances (PFASs), pesticides, microplastics (MPs), and so on. In this study, the European eel (*Anguilla Anguilla Linnaeus*, 1758) was selected to study the bioconcentration of OPs, given its physiological and behavioural characteristics, which make it vulnerable and sensitive to the presence of these substances (1). Furthermore, in 2007, it was placed on the International Union for Conservation of Nature's Red List of Endangered Species, listed as "critically endangered" (2). Overfishing, water barriers hindering migration, climate change, parasitic diseases, habitat reduction and chemical pollution, among others, can be defined as main causes of the drastic reduction of its population since 1970 (1). The general aim of this research was to study the bioconcentration and depuration kinetics of a mixture of 22 OPs in muscle, liver tissue and plasma of European eel, and to evaluate the influence of MPs in them.

## Materials and methods

The OPs included 10 PPCPs, 5 pesticides, 5 PFASs and 2 illicit drugs, which were vehiculated through water or food, depending on their chemical properties. The compounds of the former group are acetaminophen, atenolol, bentazone, bufotenine, caffeine, diclofenac, etoricoxib, ibuprofen, imazalil, naproxen, perfluorobutane sulfonate (PFBS), perfluorodecanoic acid (PFDA), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluoropentanoic acid (PFPeA), salicylic acid, terbuthylazine, and vildagliptin (n=18). The compounds that were supplied through food include (n=4): chlorfenvinphos, chlorpyrifos, triclosan and 4-methoxyphencyclidine (4-MeO-PCP). The experimental study lasted 58 days including: (a) an exposure stage (days 0-28), in which the mixture of OPs and spherical MPs (polyethylene) was administered through water (10 µg/L of OPs and 0.04 g MPs per specimen), and through food (20 ng per eel/day); and (b) a depuration phase (days 29-58), in which the water from aquariums was renewed (without OPs). OPs in eels' muscle and liver samples were extracted by QuEChERS and dispersive solid phase extraction (dSPE) whereas plasma was extracted by SPE (3). Extracts were analysed via UHPLC-MS/MS. The average weight and lengths of the silver (adult) European eels (n=144) employed were 63.3±18.3 g and 30.8±3.1 cm.

## Results and discussion

The exposure through water of diclofenac, etoricoxib, imazalil, PFDA, PFOA, PFOS, terbuthylazine, and through food of chlorpyrifos, at the tested concentrations, seemed to cause their bioconcentration in the three eels' tissues (with and without MPs). Other pollutants appeared to show a preferential tissue bioconcentration as caffeine, chlorfenvinphos (muscle), naproxen (liver), bufotenine and PFBS (plasma), triclosan (muscle, liver), and 4-MeO-PCP (liver, plasma). During the exposure stage, two different trends were observed. A first group of OPs with increasing concentrations in the three tissues (PFDA, PFOS, PFOA, chlorpyrifos, and terbuthylazine), or in some of them, and a second group with less clear or erratic trends. PFASs concentrations followed the trend plasma > liver tissue > muscle. The occurrence of MPs seemed to play an important role in the bioconcentration kinetic of the OPs analysed here. Some recent research have proved that MPs can act as vectors of pollutants in the aqueous environment (4). During the depuration stage, the OPs described two trends. In the first one, concentrations tended to decrease (chlorpyrifos, terbuthylazine), and in the second one, concentrations were similar or even tended to be higher than were those measured at the exposure phase (PFDA, PFOS, PFOA). In relation to the occurrence of MPs, concentrations reached at the end of the depuration phase tended to be higher at the group with MPs than at group without them in muscle and liver tissue, but lower in the group without them (plasma).

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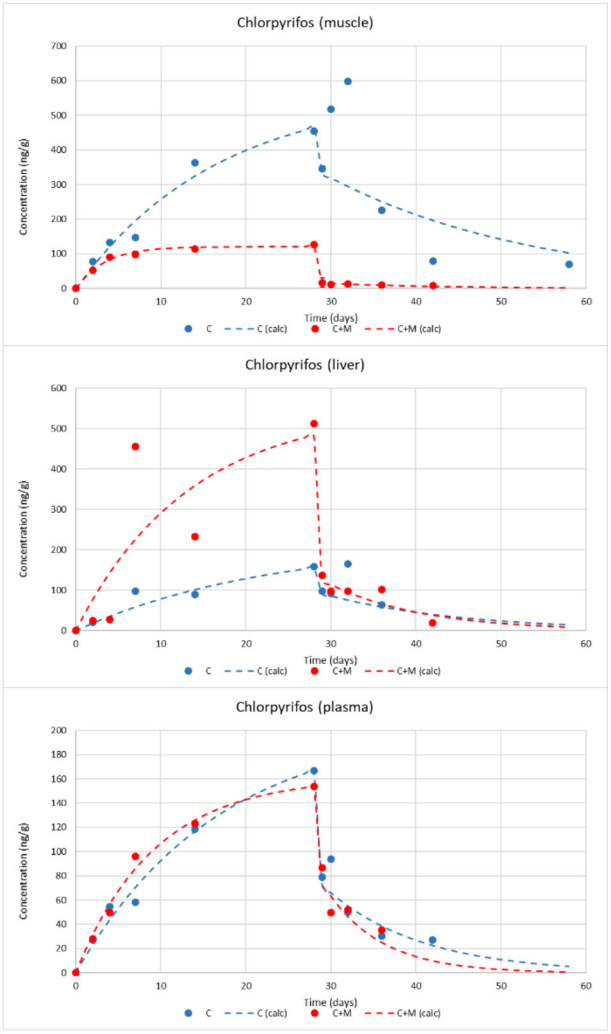


**Fig 1.** Toxico-kinetic curves from the modelling in both groups (C: OPs, C+M: OPs + MPs) for chlorpyrifos.

## Conclusions

Many pollutants are widespread and measured concentrations are at a level that more than likely is causing ecotoxicological effects in European eels. Legacy substances such as PCBs and heavy metals are relatively well studied, however the impact on the eel of emerging compounds (e.g. PPCPs, PFASs, illicit drugs, etc.) which are known to pose serious and increasing problems in aquatic ecosystems, is poorly understood. Compared to other fish species, eels are challenging to study and to protect due to their catadromous and semelparous lifecycle. We have proved that pollutants are not distributed evenly among eels' tissues and that higher concentrations can be found in plasma and liver than in muscle. Bioconcentration and depuration of OPs in eels seemed to be highly determined by the type of pollutant, and by different processes related to the combination of a mixture of chemicals including the presence of MPs. Further research is needed to clearly understand the effects and toxicity, singly and cumulatively, of multiple and different pollutants in target organs of (wild) eels, and to assess their partitioning and distribution among eel tissues.

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# *IN SILICO* MAPPING OF TRANSPOSABLE-ELEMENT FOOTPRINTS ON IMMUNE GENES ACROSS FARMED TELEOST GENOMES

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**Introduction**

Transposable elements (TEs), better known as transposons or "jumping genes", are dispersed, repetitive DNA segments able to move and insert autonomously in the genome through copy-and-paste RNA (class I retrotransposons) or cut-and-paste DNA mechanisms (class II transposons) (Finnegan, 1989). TEs account for most of the vertebrate genome and, although long dismissed as "junk DNA", they have fuelled events such as speciation, de-novo gene birth and large-scale chromosomal rearrangements (Auvinet et al., 2018; Long et al., 2003; Lönnig & Saedler, 2002). However, uncontrolled mobilisation of TEs can compromise genome integrity, and trigger chronic inflammation or even tumourigenesis through persistent activation of transcription factors such as NF-κB and p53 (Gudkov et al., 2011). In this sense, epigenetic locks such as methylation and acetylation are able to keep TEs silent (Saul & Kosinsky, 2021). In addition, the immune system is able to operate as a second surveillance mechanism whereby, when epigenetic silencing fails, TE-derived nucleic acids accumulate in the cytoplasm. Then, they are able to mimic viral signatures and activate canonical DNA/RNA sensors such as Toll-like receptors, triggering type I interferon and pro-inflammatory cytokine responses that restrain further mobilisation (Rakoff-Nahoum & Medzhitov, 2009). In this context, teleost fishes are particularly noteworthy for harbouring the largest repertoire of TEs known to date, while possessing the earliest and most fully developed combination of innate and adaptive immune defences among vertebrates (Swain & Nayak, 2009). Nevertheless, the critical relationship between TEs and immune system, potentially central to understanding TE activity and its consequences, remains largely unexplored in fish species.

**Material and Methods**

Canonical TEs consensus sequences for eight cultured teleosts—zebrafish (*Danio rerio*), green pufferfish (*Tetraodon nigroviridis*), Japanese medaka (*Oryzias latipes*), European seabass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla anguilla*), Nile tilapia (*Oreochromis niloticus*) and gilthead seabream (*Sparus aurata*)—were retrieved from FishTEDB. Each species-specific library was imported into OmicsBox software (v. 3.4.5) and launched as a BLASTn query (E-value $\leq 1 \times 10^{-10}$) against the full Actinopterygii section of GenBank. The resulting hit tables were inspected manually, and for every TE, we flagged alignments whose first (highest-scoring, longest) match overlapped a locus annotated as immune-related (e.g., *tlr*, *nlr*, *ifn*, complement, cytokine, antiviral genes, etc.) and discarded the rest to avoid redundancies produced by long consensus sequences. For each retained TE-immune pair we recorded TE class/superfamily, alignment coverage (% query length) and identity; matched immune gene names were cross-checked in UniProt to confirm their immune function. Curated lists were then collated by species to expose shared versus lineage-specific patterns.

**Results and Discussion**

The screen yielded 333 robust TE–immune contacts distributed among the eight species. Most insertions clustered in membrane-bound pathogen-recognition receptors (Toll-like, complement and assorted cytokine receptors), cytosolic inflammasome sensors of the NLR-PYD/CARD family, and antiviral effectors such as type-I interferons, and Mx proteins. hAT, Tc1-Mariner, and L2- and L1-type LINEs lineages dominated, underscoring repeated involvement of both DNA-transposon and retrotransposon and classes in shaping immune loci. Based on these results, we hypothesize that TE fragments persisting in regulatory or coding regions of membrane receptors could have been selectively retained whenever they conferred improved pathogen detection or signalling capacity, thereby driving the expansion and functional diversification of innate-immune receptors in fish (Krasnov et al., 2005). Incomplete insertions—such as the TE fragment within *tlr9* of rainbow trout—could likewise function as inducible regulatory modules, adding an extra layer of epigenetic control (Ortega-Villaizan et al., 2009). Altogether, the observed pattern could be consistent with a scenario in which TE-driven genetic variation helps teleosts adapt to pathogen-rich aquatic habitats.

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In conclusion, this multi-species *in-silico* survey uncovers a widespread presence of TE across key innate-immune pathways of eight farmed teleosts, underlining mobile DNA as a silent architect of fish immune evolution. Therefore, our results offer a new insight into the relationship between ET and the immune system that may be key to the search for genomic markers and the development of new vaccines in aquaculture.

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# ADVANCE YOUR RESEARCH THROUGH AQUASERV

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AQUASERV (aquaserv-ri.eu) is a research infrastructure (RI) project funded by Horizon Europe that offers access to scientific services in over 60 European facilities related to Aquaculture, Fisheries, Ecosystem Restoration, and the Blue Economy.

AQUASERV aims to significantly further scientific advances and promote and facilitate the implementation of the European Common Fisheries Policy, the Farm to Fork Strategy, the Sustainable Blue Economy and the European Green Deal.

AQUASERV will achieve these objectives by offering scientists from academia and business remote and on-site transnational (TA) and virtual (VA) access to an advanced set of European research infrastructures and their nodes related to research and management of marine and freshwater biological resources, food and biotechnology. These include the European Marine Biological Resource Centre (EMBRC ERIC), the Analysis and Experimentation on Ecosystems (AnaEE ERIC), the Aquaculture for Excellence for Aquaculture in Fish (AQUAEXCEL), the Infrastructure for Promoting Metrology in Food and Nutrition (METROFOOD), and the International Council for Exploration of the Sea. It also includes the Research Infrastructure for Science and Innovation Policy Studies (RISIS) to bring contributions from the social sciences, and the European Society for Aquaculture (EAS).

Detailed information on how to benefit from AQUSERV will be provided.

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# THE SUITABILITY OF THE GUADALQUIVIR ESTUARY TO DRIVE THE REHABILITATION OF DRAINED MARSHES AND ACCESS NEW NET SOURCES OF AQUATIC FOOD

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**Introduction**

The Guadalquivir marshes (SW Spain) have been the subject of a vast transformation for agricultural purposes since the 1940s, affecting ~75% of their original surface area of around 2,000 km$^2$. The affected areas were hydrologically disconnected from the estuary, which has also been adapted as a navigation channel, showing high turbidity, low chlorophyll content, and a high nutrient load. A significant fraction of the transformed marsh, particularly on the left bank of the estuary, remains uncultivated. Recently, initiatives have been undertaken to reconnect some of these uncultivated areas of the marsh with the estuary. Within this context, a new situation arises in which a brackish, hypereutrophic estuarine environment is uninhibited in its light limitation when it enters shallow-water marshes. Controlling the residence time of these estuarine water bodies in marsh ponds allows for the development of significant primary productivity, which provides the trophic support for obtaining new aquatic food resources on a net basis. Thus, the goal of present study was to understand the potential for primary food production in marshes flooded with brackish water from a eutrophic estuary.

**Material and Methods**

The study was conducted in a 13.5-hectare rehabilitated marshland (36º53'34.41"N; 6º13'41.23"W), flooded with water from the Guadalquivir estuary. Water flows from those ponds (1.2 m depth) in this area used for extensive aquaculture to another bigger pond (0.5 m depth) that is managed to promote wildlife conservation. Nutrients ($NO_3^-$, $NO_2^-$, $NH_4^+$, $PO_4^{3-}$, $SiO_4^{4-}$) and chlorophyll a were determined for an annual cycle during the 2023-2024 biennium. The species composition of phytoplankton was also determined by microscopic observation. Sampling was monthly in the estuary and fortnightly in the marsh ponds. To determine primary production rates, dissolved oxygen and pH were recorded every 10 min. The autonomous HOBO U26-001 (oxygen) and HOBO MX2501 (pH) probes were used, which were replaced and recalibrated during the fortnightly sampling periods. The recorded dissolved oxygen series were integrated according to Needoba et al. (2012) to calculate the corresponding gross (GPP) and net primary production (NPP) rates. To determine GPP and NPP directly from daily changes in dissolved inorganic carbon (DIC), the thermodynamic dissociation constants of Millero (2010) were used for the specific conditions of total alkalinity, pH, salinity and temperature in the water.

**Results**

The main feature of the eutrophic nature of the Guadalquivir Estuary was its high nitrate concentration. It is interesting to note how, after flowing through the marsh ponds, the level of nitrate remaining in the water was negligible, while silicate, which also had a high concentration in the estuary, was only reduced by around 50% (Table 1). The significant ammonium increase in marsh waters (Table 1) is consistent with its increased metabolic activity. Around 35% of the relatively lower phosphate concentration in the estuary, that resulted in a high molar N/P ratio around 65, remained in marsh ponds (Table 1).

Salinity at the estuary water intake point ranged from 12.7 to 25.9. Chlorophyll a concentration increased from an average of 5.9±3.8 µg l$^{-1}$ in the estuary to 75.4±44.1 µg l$^{-1}$ in the marsh. The phytoplankton community in the marsh ponds was dominated by high nutritional value microalgae species of the haptophye (mainly *Diacronema* spp) and diatom groups (*Chaetoceros*, *Cylindrotheca*). Both GPP and NPP were highest in spring and summer, decreasing by approximately half in autumn and winter (Figure 1). NPP accounted for 63% or 50% of GPP when primary production was calculated according to changes in dissolved oxygen or DIC, respectively. Estimates based on DIC changes were lower than those based on oxygen evolution (Figure 1), indicating a significantly higher ($P<0.05$) photosynthetic quotient (PQ) in summer (PQ=1.84) than in the other seasons (PQ=1.27 in winter, PQ=1.31 in spring, PQ=1.47 in autumn).

*(Continued on next page)*

Table 1. Annual records for the mean and standard deviation of nitrate, nitrite, ammonium, phosphate and silicate concentrations (µM) in the Guadalquivir estuary and after flowing through the studied marsh ponds.

| | $NO_3^-$ | $NO_2^-$ | $NH_4^+$ | $PO_4^{3-}$ | $SiO_4^{4+}$ |
|---|---|---|---|---|---|
| Estuary | 131.7±56.1 | 1.1±1.1 | 1.8±1.9 | 2.0±0.7 | 70.6±64.2 |
| Marsh | 0.1±0.6 | 0.8±1.1 | 14.3±18.5 | 0.7±0.6 | 41.2±17.0 |

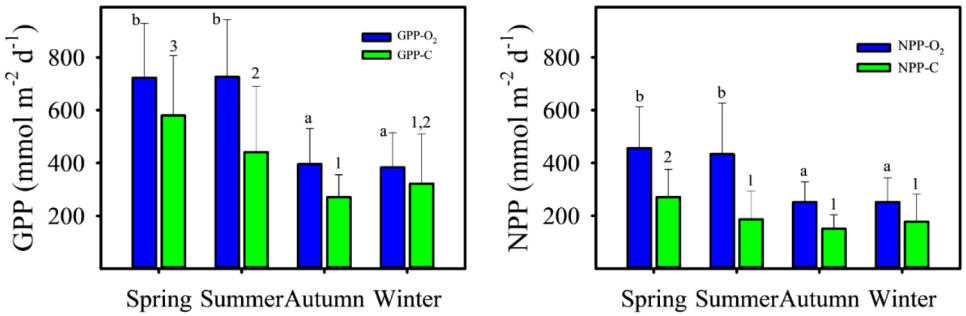


Figure 1. Seasonal gross (left graph) and net (right graph) primary production in marsh ponds flooded with Guadalquivir estuary waters. Same letters and numbers indicate homogeneous seasonal groups (Tukey test; $p<0.05$) based on dissolved oxygen or DIC evolution, respectively

In terms of net C, the average daily primary production for the annual cycle was 187±86 mmol C $m^{-2}$ $d^{-1}$. By integrating the daily NPP records for an annual cycle and transforming to units of g of C per hectare, a value of 8190 kg C $ha^{-1}$ $y^{-1}$ was obtained for the study area. This would be the maximum amount of C usable by the consumers being exploited.

**Discussion**

The result obtained for NPP was very similar to that described by Thébault et al (2008) for reconnected salt ponds in San Francisco Bay, where nutrient load was also high. From the results of these authors, a trophic transmission efficiency from NPP of 3% for planktivorous fish and 14% for invertebrates can be inferred. These coefficients would correspond to respective maximum potential production values of 245 and 1146 kg C $ha^{-1}$ $y^{-1}$. Assuming a 40% C content in fishmeal (FM), the above figures would approach to 612 and 2865 kg FM $ha^{-1}$ $y^{-1}$, respectively. However, in this type of aquatic system, losses of natural production are significant, particularly those due to predation by wildlife. Therefore, to optimize the production of aquaculture food resources, it is necessary to develop specific management practices, implementing different ecosystem services in the economic balance of the activity. Land availability is assumed to be high since a recent inventory of 90,800 $km^2$ of preserved tidal marshland worldwide (Murray et al., 2022) suggests that over 280,000 $km^2$ of marshland has been altered in some way. It can be concluded that rehabilitating marshes fringing eutrophic estuaries and using simple, unfed aquaculture practices based on water management is a practical option to provide important and recognized ecosystem services such as access to new net aquaculture food resources of high nutritional quality, removal of excess estuarine nutrients to prevent coastal eutrophication and trophic support for wildlife preservation.

**Acknowledgements**

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# ENSURING EU-METAL(OIDS) LEVELS BELOW REGULATORY LIMITS FOR CONSUMERS IN GILTHEAD SEABREAM (*Sparus aurata*)

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**Introduction**

Marine aquaculture production relies primarily on wild-caught fish as feed ingredients such as fishmeal (FM) and fish oil (FO). These key ingredients are generally recognised as highly valuable as they meet nutritional needs for fish growth, being easily digestible and containing high levels of long-chain polyunsaturated fatty acids (PUFA).

The levels of contaminants and nutrients in fish from aquaculture and wild-caught sources are a timely and relevant issue for food safety. Contaminants such as heavy metals and metaloids (metal(oids)), PCBs, DDT, and PAHs pose toxic risks due to bioaccumulation, while nutrient levels like Se, Zn, and Fe vary depending on diet and habitat. After a comparison between aquaculture and wild fish in terms of metal(oids), other contaminants, and nutrients, due to the difference in their environmental conditions, feed and origin, it can be said that higher concentrations of some metal(oids) were observed in wild fish than in aquaculture as well as in other contaminants. The mean Hg and As concentration in wild fish was 0.06 and 3.26 μg/g, respectively, while in aquaculture fish was 0.038 and 1.23 μg/g. The DDT mean concentration in wild fish was 196.48 ng/g, while in aquaculture fish was 44.64 ng/g. The difference in these concentrations does not only depend on the production system, other factors such as age, physiological state or the degree of environmental pollution can have a considerable effect. Therefore, there is a stronger trend toward replacing FM rather than FO because protein alternatives like insect meal and poultry by-products are more nutritional, accessible, like FM and cost-effective. Another critical motivation for seeking alternative ingredients is the reduction of metal(oids) in farmed fish. By incorporating alternative protein sources with lower contamination levels, it is possible to enhance the safety and quality of aquaculture products while maintaining sustainable production practices.

Taking all mentioned above into account, the purpose of this research was to explore the effect of feeding with different alternative proteins (compared to fishmeal) on the composition metal(iods) in Sparus aurata fillets at two growing stages (300 and 800 g).

**Materials and methods**

Three types of feed formulations (n=3, CTRL: fish meal-based diet, PAP: processed animal protein-based diet, and ALT: alternative protein-based diet). Fish sampling took place at two growth stages: when fish reached 300 g and 800 g. The nomenclature for fish was as follows: i) individuals reached 300 g: CTRL300, PAP300 and ALT300; and, ii) individuals reached 800 g: CTRL800, PAP800 and ALT800. Inductively coupled plasma mass spectrometry (ICP-MS) was used for quantification of metal(oids). 0.5 g of freeze-dried sample was weighed into the Teflon cups of the microwave digester, 7 mL of $HNO_3$ 69% ppt quality were added, the tubes were left open for 1 hour pre-digestion and 2 mL of $H_2O_2$ 30% ppt quality were added. HPLC-ICP-MS (Shimadzu, Japón) was used to analyse speciation of As: AsIII (inorganic arsenite), AsV (inorganic arsenate), AsB (arsenobetaine), AsC (arsenocholine), DMA (dimethylarsinic acid) and MMA (monomethylarsonic acid).

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**Results and discussion**

Regarding metal(oids), the median and mean values were similar in most cases. Overall, our results align with findings in the literature that highlight the influence of fish size and feed formulation could affect the accumulation of them. As concentrations were higher in 800 g fish than in 300 g fish (1.5 times more in CTRL800, 1.3 times more in PAP800, and 1.6 times more in ALT800), with significant differences observed between formulations. Specifically, CTRL showed the highest concentrations, with FM800 having 3.7 times more As than the other diets, and CTRL300 having 3.3 and 4.0 times more than PAP300 and ALT300, respectively. Among As species, AsB was the unique species detected in samples. Recently, the European Commission asked EFSA for a risk assessment on complex organoarsenic species in food. They are typically found in marine foods and comprise mainly arsenobetaine (AsB), arsenosugars and arsenolipids. Following with the rest of the contaminants and potentially toxic elements, Hg levels were also higher in 800 g fish compared to 300 g fish (3.6 times more in CTRL800, 5.1 times more in PAP800, and 6.8 times more in ALT800). While no significant differences were found between formulations, a trend was observed with FM showing higher levels than PAP and ALT. Cd content was generally higher in 800 g fish (2.8 times more in CTRL800, 3.7 times more in PAP800, and 4.4 times more in ALT800), although the median was lower than the mean in 800 g fish across all formulations. Pb concentrations were higher in 800 g fish compared to 300 g fish, though no significant differences were observed. It is noteworthy that the median and mean values were not similar for PAP800 and ALT800. Overall, 800 g fish had considerably higher Pb concentrations than 300 g fish, with increases of 18.9 times in FM800, 18.6 times in PAP800, and 17.1 times in ALT800).

**Conclusions**

The differences between formulations, particularly in As and Hg levels, emphasize the need for a careful selection of feed ingredients to minimize metal(oids) exposure in aquaculture. Further research is needed to better understand the mechanisms governing these compounds metabolism and to evaluate the potential health risks associated with their accumulation in farmed fish.

# OBSERVATION OF ANEMIA IN POOR-PERFORMING RAINBOW TROUT (*Oncorhynchus mykiss*) WITH NEPHROCALCINOSIS IN HATCHERIES

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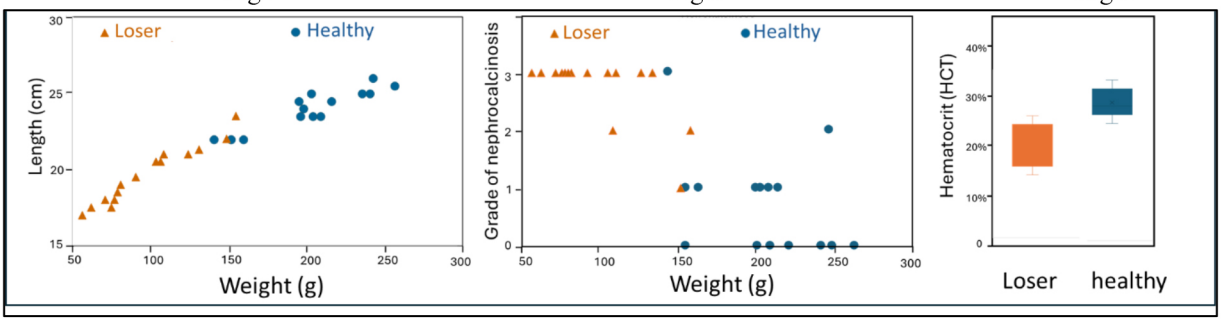
**Introduction:**
Nephrocalcinosis has become a growing problem in Norwegian hatcheries for salmonids. The disease is considered an important cause of reduced welfare, reduced growth and increased mortality. Poor-performing fish, often referred to as "losers," also represent a significant problem in the hatchery phase in salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss) farming. The underlying causes are multifactorial and remain poorly understood.

The primary aim of this study is to investigate histological and hematological changes in rainbow trout (Oncorhynchus mykiss) during the hatchery phase, with the goal of identifying factors associated with nephrocalcinosis and the development of the loser phenotype.

**Material, methods and results:**
Kidney and blood samples were collected from 15 loser fish (98 ±31 g) and 15 apparently healthy fish (201 ± 36 g) from local hatcheries (Fig. 1). Bleeding was not detected in any of the fish. Histological evaluation revealed well-developed nephrocalcinosis in all loser fish, corresponding to grade 2 or 3 lesions. Among the healthy fish, 6 of 15 displayed mild mineral precipitation and dilation of Bowman's capsule. Additionally, two individuals exhibited grade 2 or 3 nephrocalcinosis, despite being classified as healthy (Fig. 2). Hematological analysis revealed a significantly lower hematocrit (HCT) in loser fish (19 ± 4%) compared to healthy individuals (29 ± 3%; $p < 0.05$; Fig. 3). Plasma cortisol levels were also markedly elevated in loser fish (17.9 ± 7.3 ng/mL) relative to healthy controls (4.4 ± 2.5 ng/mL, $p < 0.001$). However, plasma glucose concentrations did not differ significantly between the groups.

**Conclusion:**
These results indicate a strong association between advanced nephrocalcinosis, reduced hematocrit, and the loser phenotype. Impaired excretory function and anemia may contribute to the syndrome's development. To further explore causative mechanisms, subsequent analyses will include comprehensive blood chemistry profiling, stress biomarker evaluation, and measurements of erythropoietin levels. In the next phase, we will longitudinally monitor the progression of nephrocalcinosis, loser syndrome, and hematopoietic disturbances from early juvenile stages (20 g) up to 200 g. These observations will be correlated with detailed renal histopathology, and the potential influence of seawater transfer and vaccination will also be assessed.

Fig 1 Fig 2 Fig 3



# VALIDATION OF DIRECT MEASUREMENT OF FECAL CORTISOL METABOLITES IN ATLANTIC SALMON (*Salmo salar*) USING A COMMERCIAL ELISA KIT

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**Introduction:**
Atlantic salmon farming represents a cornerstone of Norway's aquaculture sector, with a production output of 1.6 million tons in 2021. Ensuring high standards of fish welfare is essential to support the industry's sustainable development. Compared to traditional plasma cortisol measurements, the assessment of fecal cortisol and its metabolites (FCMs) offers a less invasive and more efficient approach for evaluating the health and welfare of salmonids. This method not only reduces handling related stress but also supports long-term sustainability in aquaculture practices.

The primary aim of this study is to evaluate the performance of four commercial ELISA kits for quantifying FCMs in Atlantic salmon (*Salmo salar*), and to further investigate the distribution of FCMs across the liquid, solid, and homogenized total fractions of fecal samples.

**Material and methods:**
Atlantic salmon used in this study were reared at a commercial fish farm located on the northwestern coast of Norway. Fecal samples were collected both prior to and 24 hours after sea lice counting procedures conducted in a commercial sea cage. Samples were obtained via stripping and partially subjected to centrifugation to separate liquid and solid fractions. FCMs were analyzed using two methods: enzyme-linked immunosorbent assay (ELISA) and liquid chromatography–tandem mass spectrometry (LC–MS/MS).

**Results:**
Biological validation demonstrated that both the Cayman and Neogen kits detected biologically meaningful changes in FCMs following stress induced by sea lice counting procedures and handling. Analytical validation demonstrated high accuracy, reliability and reproducibility of both kits. The specificity test revealed that the Neogen kit exhibited relatively higher cross-reactivity to cortisol and its metabolites compared to the other kits. Cortisol and four of its metabolites (5β-THF, cortisone, 5β-DHE, 5β-THE) have previously been identified as reliable stress biomarkers in farmed Atlantic salmon. In this study, all five compounds were successfully detected in the total, liquid, and solid fecal fractions using LC–MS/MS. Analysis revealed no significant differences in the concentrations of FCMs across the three fecal components (Fig.1). Strong correlations were found among the sample types ($p<0.05$), indicating consistent distribution of these biomarkers. Similar patterns were observed when concentrations were measured using ELISA, with values across the fecal fractions remaining consistent (Fig.2). FCM concentrations determined via ELISA showed a strong positive correlation with LC–MS/MS measurements ($p<0.05$), although the LC–MS/MS technique consistently produced higher values ($p<0.05$). Moreover, the FCM concentrations measured directly from the liquid fecal fraction using ELISA without prior extraction correlated well with the results obtained from extracted samples ($p<0.05$; Fig.2).

**Conclusion:**
In conclusion, these findings emphasize the need for thorough validation of ELISA kits prior to their application in stress monitoring. Among the kits tested, the Neogen ELISA kit demonstrated reliable performance for quantifying FCMs in Atlantic salmon. The study also confirmed that FCMs are uniformly distributed between the liquid and solid components of fecal samples. While LC–MS/MS remains the more precise and sensitive method for FCMs analysis, ELISA provides a practical and accessible alternative for routine use in most laboratories. Notably, FCM concentrations measured by direct ELISA analysis reflected those obtained using LC–MS/MS, supporting its utility for non-invasive stress assessment in aquaculture settings.

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Fig. 1

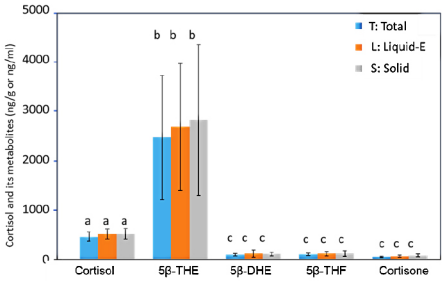
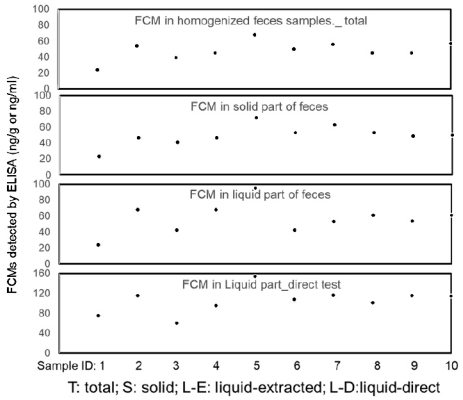

Cortisol and its metabolites (ng/g or ng/ml)
5000
4000
3000
2000
1000
0
T: Total
L: Liquid-E
S: Solid
a a a
b b b
c c c
c c c
c c c
Cortisol
5β-THE
5β-DHE
5β-THF
Cortisone

Fig. 2


FCMs detected by ELISA (ng/g or ng/ml)
FCM in homogenized feces samples_ total
FCM in solid part of feces
FCM in liquid part of feces
FCM in Liquid part_direct test
Sample ID: 1 2 3 4 5 6 7 8 9 10
T: total; S: solid; L-E: liquid-extracted; L-D:liquid-direct

# MACROALGAE PRODUCTION IN THE UK - FROM WILD HARVEST TO AQUACULTURE

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**Introduction**
In the UK, macroalgae or seaweeds have been harvested from the wild for centuries and traditionally used for food, feed and fertilizers. Recently, seaweed biomass has been used to produce bio-stimulants, nutraceuticals, cosmetics, biofuel, bioplastic and biomaterial. An initial attempt to commercially farm seaweed in the 1980s-90s was not sustained. Over the last decade, a resurgence of interest in seaweed has led to the re-development of commercial seaweed aquaculture in the UK. However, no data or centralised information are available on current UK seaweed production from aquaculture or wild harvest.

**Methods**
A combination of literature review, web searches and interviews with seaweed producers, seaweed organisation and regulatory bodies in the UK has been used to determine the status of the UK seaweed industry, including estimates of current production (from both wild harvest and aquaculture), number/extent of farms, and species cultivated.

**Results and discussion**
The seaweed industry is growing in the UK, as the number of farms, seaweed-related businesses and marketed seaweed-products have increased since a previous study in 2016. Species harvested in the UK include brown seaweed (e.g. *Saccharina latissima*, *Laminaria digitata*, *Alaria esculenta*), red seaweed (*Palmaria palmata*) and green seaweed (*Ulva* spp.). Farms use various techniques (longline, droppers, etc.) to suit location, farm size, end uses and local environmental conditions. Growth of the UK industry is hindered by multiple issues, also common to Europe: obtaining licences and investment for prospective farms; lack of standards for farming and products; technological barriers; the need for social licence to operate; spatial planning. It is important to document current activities, so operational lessons (e.g. unproven niche markets; economies of scale and infrastructure; competition from wild harvest and imports) can be learnt by this emerging aquaculture sector.

# EFFECTS OF PROBIOTICS, PREBIOTICS, AND SYNBIOTICS ON TILAPIA (*Oreochromis niloticus*) GROWTH AND IMMUNE RESPONSE IN CONTROLLED AQUACULTURE SYSTEMS

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This study investigates the effects of probiotics, prebiotics, and synbiotics on the growth and immune response of Nile tilapia (*O. niloticus*) across various aquaculture systems, based on a systematic review and meta-analysis of the existing literature. The results reveal significant improvements in growth, especially in closed systems like Biofloc and Recirculating Aquaculture Systems (RAS), where microbial stability and prolonged interactions between bioactives and fish are crucial for growth performance. While prebiotics and synbiotics also showed positive effects, their application was less frequent than probiotics, and their field applicability (at production scale) remains inconclusive. Probiotics, on the other hand, positively influenced key immune components, including TLR expression, lymphocyte differentiation, phagocytic activity, and antibody production. Despite these promising results, the study highlights a lack of understanding regarding immune responses in different aquaculture systems, stressing the need for more standardized and comprehensive immune assessments. To advance this research, it is essential to explore the molecular mechanisms behind these effects, particularly using advanced transcriptomic profiling (RNA-seq), to gain a deeper understanding of how bioactives interact with fish immunity. Additionally, gene expression following pathogen challenge assays with pathogens other than *A. hydrophila* should be included, with a focus on emerging diseases of both scientific and practical interest. Applying these findings in commercial-scale aquaculture systems will help integrate these treatments to improve fish health and productivity under real-world farming conditions.

# SPATIAL MANAGEMENT FOR AQUACULTURE PLANNING: DEFINING NEEDS FOR ASSISTING COUNTRIES

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The sustainable development of aquaculture increasingly depends on the capacity of countries to integrate sectoral growth of blue economies within a marine spatial planning (MSP) integrated process. Aquaculture has the potential to contribute to food security, coastal livelihoods, and the achievement of the Sustainable Development Goals/Agenda 2030 (Troell *et al.* 2023). However, its expansion often competes for space with other maritime activities and requires a science-based approach to identify suitable areas to be allocated to this activity, minimizing conflicts with other uses. In many regions, MSP has not yet been applied to aquaculture, or only partially introduced, leaving countries without the tools needed to plan for sustainable sectoral growth.

In this context, ISPRA and FAO co-organized in October 2024 the expert workshop "Spatial management for aquaculture planning: Defining needs for assisting countries", held at FAO headquarters in Rome. The workshop brought together 18 experts from different regions of the world, representing diverse experiences and perspectives, to define priorities and outline a roadmap of actions for supporting countries in advancing aquaculture spatial planning. The initiative was motivated by the recognition that knowledge gaps, limited data availability, fragmented governance, and lack of institutional capacity are among the key challenges delaying the integration of aquaculture into the broader MSP processes.

The roadmap outlined during the workshop identifies strategic objectives and actions across five priority areas: (i) governance and institutional frameworks, focusing on streamlined interactions between authorities; (ii) knowledge support for spatial planning in aquaculture, including data, innovative tools, and smart aquaculture; (iii) criteria and guiding elements for allocation of space, co-use of marine areas, land–sea interactions, and adaptation to climate change; (iv) capacity building, awareness, and stakeholder engagement through training and digital platforms; and (v) marine conservation and restoration, emphasizing how spatial planning in aquaculture can contribute to ecological objectives and conservation targets. These directions reflect the need for both technical and policy support, highlighting the role of international cooperation in sharing experiences and mobilizing resources.

The workshop was also aligned with Action XIV of the Updated Joint Roadmap to accelerate Marine/Maritime Spatial Planning processes worldwide (2022–2027), which calls for co-producing sector-specific recommendations on MSP, including aquaculture.
In order to contribute to the development of MSP in the aquaculture sector within the broader MSPglobal framework, the workshop outcomes aim to contribute to the international agenda of extending spatial planning to at least one third of global maritime areas under national jurisdiction by 2030 (IOC-EC 2022). The tools and outputs developed through the workshop are also coherent with the FAO Blue Transformation roadmap (2022–2030), which emphasizes effective planning, governance, and cooperation as essential to sustainable aquaculture development and to the integration of the sector into cross-sectoral policies (FAO 2022).

This presentation provides insights into the rationale for convening the workshop, the process of building consensus among international experts, and the expected outputs in terms of guidance and actions. It also reflects on how this roadmap can serve as a practical contribution, helping countries in integrating aquaculture into MSP in a sustainable way that is sustainable, with a science-based approach, and consistent with global ocean governance objectives.

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# MUSSELS IN AQUACULTURE AS BIOSENSORS OF HEAT STRESS: PARAMETERIZATION IN THE LABORATORY

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## Introduction

Despite the socio-economic and environmental importance of the mussel farming sector in Spain, there are currently no monitoring or early warning systems in place to establish mitigation measures against extreme conditions, often exacerbated by climate change.

In this context, the main objective of the project MoMeNTo is to develop an early warning system based on the use of mussels as biosensors, enabling the direct integration of environmental variations into the physiological state of cultivated mussels. To achieve this, a low-cost valvometry sensor developed by CSIC is being used to monitor and characterize valve gaping behaviour as an indicator of thermal stress events, jointly with other classical indicators of physiological stress.

As a first step, a laboratory experiment was developed to relate valve behaviour with other indicators of stress (clearance rate, tenacity of byssus attachment, activity of biochemical biomarkers, histopathological condition), taking also into account the origin of the mussels (Mediterranean or Atlantic) to evaluate different temperature thresholds or strategies to fight heat stress according to their former life-time ecological background.

## Material and Methods

One tank (25 L) with 33 individuals per temperature treatment (8 levels: 16, 18, 20, 22, 25, 27, 29 and 31ºC), and origin (2 levels: Mediterranean and Atlantic) were maintained for 15 days at the target temperatures after an acclimatation ramp of 2ºC/day. Valve behaviour was recorded continuously (10 Hz) during the experiment using hall sensors and a low-cost system developed by IIM-CSIC (Gilcoto et al., 2023). At the end of the exposure time a series of variables were also measured, including condition index (CI), clearance rate (CR), tenacity of the byssus attachment (TEN), histopathological condition and the activities of 5 biomarkers of physiological stress.

## Results and discussion

Preliminary results show common patterns between origins (Atlantic and Mediterranean) on the effect of temperature. Byssus attachment tenacity (TEN) and condition index (CI) showed a decrease as temperature increased, which becomes sharper for temperatures over 25ºC. Some biomarkers, as the activity of the Electron Transporter System (ETS) showed a bell-shaped relationship with temperature and a sharp decline as temperature surpasses the 25ºC, suggesting some kind of threshold at this temperature. Changes in the ETS activity and energy reserves can be related to the activation of the respiratory chain due to an increase in energy costs associated to stress (Andrade et al., 2018).

With regard to their feeding activity, although Clearance rate (CR) also showed an inverse relationship with temperature for mussels from both origins, the Atlantic mussels kept CR values almost constant until temperature surpassed 25ºC (Figure 1A), while mussels from the Mediterranean showed an inverse linear relationship with temperature (Figure 1B). Nonetheless, since CR values were greater for Mediterranean mussels than for the Atlantic ones (5.50± 0.21 and 3.84 ± 0.15 $Lh^{-1}$ respectively), the ability to feed under stress was less compromised in the Mediterranean mussels even at high temperatures. Similar results were previously observed by Lassoued et al., (2021) suggesting the relevance of original habitat conditions on the ability of mussels to adapt.

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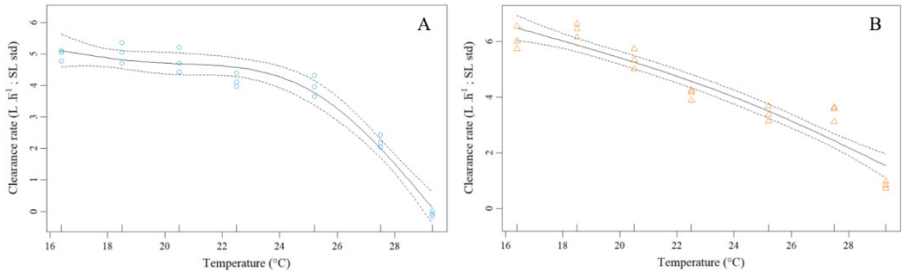


**Figure 1.** CR response to temperature for mussels coming from the (A) Atlantic and the (B) Mediterranean.

Behaviour was also altered by temperature, changing valve opening amplitude, opening time and circadian cycles (Fig. 2). Relationships between valvometry and other physiological indicators of stress are being analyzed to develop machine learning algorithms for the automatic detection of thermal stress episodes based on mussel behaviour.

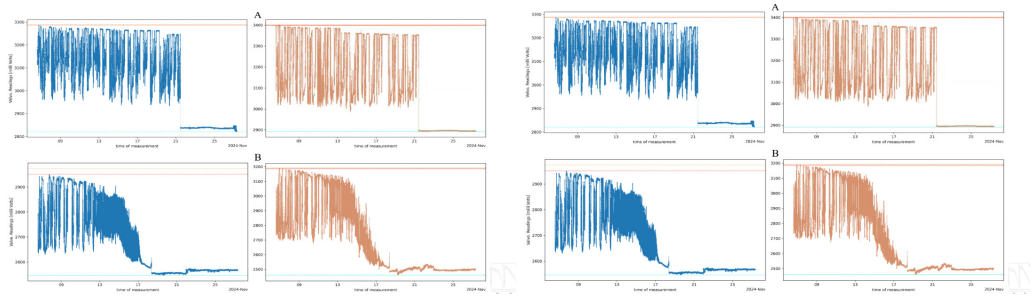


**Figure 2.** Example of the valvometry output from tanks kept at (A)18°C and (B) 31°C.

**Acknowledgements**

This research has been conducted within the framework of the MoMeNTo project, undertaken in collaboration with the Fundación Biodiversidad, from the Ministry for Ecological Transition and the Demographic Challenge, through the Pleamar Program, and is co-financed by the European Union through the EMFAF (European Maritime, Fisheries and Aquaculture Fund).

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# A DIETARY PHYTOGENIC NUTRACEUTIC IMPROVES LIPID METABOLISM AND NUTRITIONAL PROFILE IN SEABASS (*Dicentrarchus labrax*) REARED AT HIGH STOCKING DENSITIES

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## Introduction

The stocking density is a fundamental aquaculture variable with a huge impact on fish growth performance and welfare. High stocking conditions normally trigger a stress response, and it has been shown that it can decrease flesh quality and fish nutritional value (Zhang et al., 2023). The use of essential oils and phytogenic extracts as dietary additives have been reported to ameliorate fish nutritional quality in terms of fatty acid content (Sáez et al., 2024; Sutili et al., 2018). This study assesses the effectiveness on lipid metabolism and nutritional profile of the phytogenic prototype with sedative properties, FishEase-C (FEC, TecnoVit-FARMFaes Ltd.), in the diet of *Dicentrarchus labrax* juveniles reared at different stocking conditions.

## Materials and methods

Seabass juveniles ($n$ = 438, $w_i$ = 74 ± 18 g, mean ± SD) were placed into 500 L-tanks and distributed in four different experimental groups in triplicate, defined as the combination of feeding (control or FEC diet, 1 mL per kg of feed) and initial stocking density (low density: 4 kg/m$^3$ or high density: 18 kg/m$^3$). Fish were maintained for 70 days under these conditions being weighted and measured periodically. At the end of the rearing period, animals were sampled. Liver was excised to assess changes in intermediary metabolism. Muscle biopsies were taken to assess lipid classes, fatty acid profile, and enzymatic activities from intermediary metabolism. The expression of some target genes of interest were determined in both tissues.

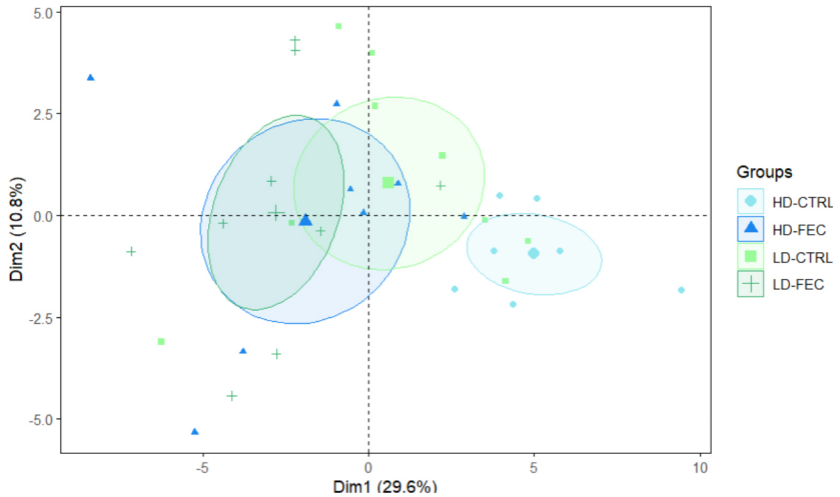


**Figure 1**. Results of the Principal Components Analysis in variables related to the muscle metabolism.

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**Results**

At end of experimental period, fish almost doubled their weight, but no significant differences on growth parameters were observed between groups. The use of the additive did not induce any negative consequences on fish. The carbohydrate and amino acid metabolism were not affected by the use of FEC prototype, nor by the stocking densities both at liver and muscle of seabass juveniles; however, some changes on lipid metabolism and nutritional composition were determined.

High stocking densities increased hepatic lipid metabolism and mobilization of triglycerides to be used as energy substrate, as it was shown by related enzymatic activities analysed (GPDH, ACL, HADH) and expression of target genes (*apoa1*, *fasn*, *lpl* and *ppar*). The use of the phytogenic prototype does not seem to ameliorate this response in liver.

In the muscle, the main lipid classes were not modified because of density conditions or addition of FEC, being triglycerides the most abundant one; however, the use of FEC reduced muscular total lipid accumulation in animals reared at low density. In addition, GPDH muscle activity decreased by the supplementation of the FEC in fish both at high and low densities, suggesting that triglycerides are not being used as an energy substrate as much as in animals fed with control diet. The fatty acids profile was also modified by stocking densities and use of dietary FEC. This use increased total n-3 polyunsaturated fatty acids (PUFAs) at both densities. It was remarkable the docosahexaenoic acid (DHA) enhancement, at both low and high concentrations, in comparison with specimens fed with control diet. Fish reared at a low density with supplemented diet increased eicosapentaenoic acid (EPA) respect to control animals. Finally, the use of FEC attenuated the decrease in the DHA/EPA ratio induced by high stocking densities.

A multivariate analysis (PCA) was performed to unravel hidden patterns in muscle metabolism (Figure 1), including enzymatic activities and fatty acids profiles. The use of dietary FEC modulates the effect of high stocking density on muscle metabolism, as a differentiation was observed between high stocking density control group and the other three experimental conditions. To conclude, these results support that dietary FEC phytogenic prototype can improve lipid management and nutritional profile in seabass, especially under chronic stress conditions by high stocking density.

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# DIETARY PHYTOGENIC ADDITIVE MODULATES INFLAMMATION AND LIPID PROFILE ALTERATIONS INDUCED BY STOCKING DENSITY IN GILTHEAD SEABREAM (*Sparus aurata*)

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**Introduction**

Intensification of aquaculture production often comes linked to increased stocking densities; however, it has been identified as one of the main stressors in the aquaculture industry, increasing the risk of health issues, altering fish behaviour, and causing growth problems. It also has an impact on muscle quality, which is determinant for product quality. *Cannabis sativa* (hemp) has been widely used in humans for its effects as an anxiolytic and anti-inflammatory. The need to find natural products to enhance fish welfare and production efficiency has led to the study of dietary hemp inclusion into fish aquafeed to reduce stress and improve immune status. Several studies in fish suggest that the use of cannabidiol decreases the expression of pro-inflammatory factors, reduces aggressiveness and improves muscular lipid profile (Wang et al., 2023; Bielawiec et al., 2023). This study assesses the effect of a phytogenic additive based on non-psychoactive *C. sativa* (CSNPE) administered through diet on the inflammation process and fatty acid profile in gilthead seabream (*Sparus aurata*) juveniles reared at high stocking densities.

**Materials and methods**

Gilthead seabream juveniles (*S. aurata*) were randomly distributed in 12 tanks (450 L), in a RAS system, establishing four different experimental groups defined as the combination of feeding (control or CSNPE) and the initial density (low stocking density, LSD): 8.5 kg/m$^3$ or high stocking density, HSD): 42 kg/m$^3$). After the 70 days, animals were sampled. Plasma was taken to assess the lipid inflammatory mediator profile; head kidney and liver samples were excised to analyse gene expression markers associated with the inflammatory process, and liver and muscle samples collected to analyse the fatty acid profile.

**Results and discussion**

A PERMANOVA analysis was performed to assess the effect of the inclusion of CSNPE in the aquafeed and the density on plasma lipid mediators. The feed supplementation induced significant changes on plasma EPA derived lipid mediators (Figure 1) (at both stocking densities), and a decrease in 12-HEPE, 15-HEPE and PGE3 were reported in animals fed with the CSNPE diet, with these eicosanoids ascribed as pro-resolving and anti-inflammatory. These results could be due to the fact that in the absence of an acute inflammatory process, there is no requirement for anti-inflammatory lipid mediators in plasma. Additionally, HEPE lipid mediators are substrate dependant, so their reduction might be associated to the *C. sativa* inclusion and its effect on cell recruitment as well as the fatty acids inclusion on lipid membranes. No significant differences were observed for *alox5* and *alox12* gene expression in liver or head kidney, which code for lipoxygenase enzymes required for eicosanoid biosynthesis. At hepatic level, the use of the supplemented diet under LSD conditions increased the *ep4* expression, which is stimulated by prostaglandins and exerts a protective role (Montero et al., 2019).

In liver, decreased levels of EPA, DHA and total polyunsaturated (PUFA) content were found in animals reared at HSD. These changes were attenuated using the CSNPE additive. In muscle, reduction of total n-3, n-6 fatty acids and PUFAs levels were found in animals reared at HSD, however, the additive did not improve the fatty acid profile, and no significant differences were found on the supplemented animals regarding control ones.

Overall, these findings suggest that CSNPE feed supplementation can influence lipid mediator dynamics and partially mitigate the physiological impact of high stocking density in the gilthead seabream.

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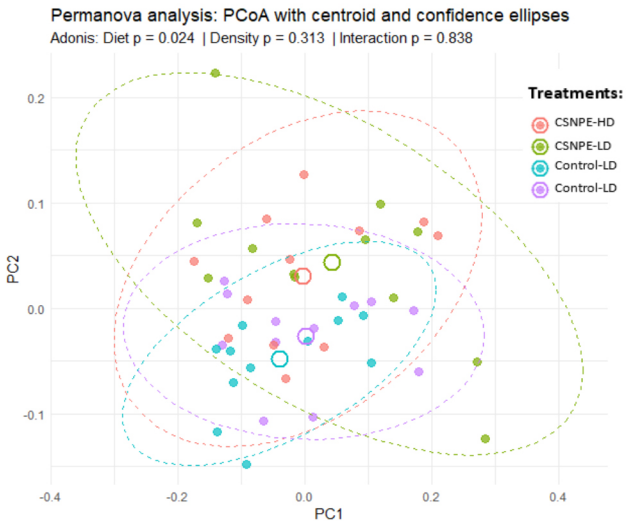


**Figure 1.** Principal Coordinates Analysis (PCoA) for EPA derived lipid mediators and PERMANOVA analysis results. Small dots represent the value obtained for each fish; large dots represent the average of each experimental group; confidence ellipses of each experimental group are also shown. For the multivariate analysis, $p < 0.05$ was considered significant.

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# EVALUATION OF FUNCTIONAL DIETARY SUPPLEMENT TO ENHANCE THERMAL RESISTANCE OF EUROPEAN SEABASS *(Dicentrarchus labrax)*

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## Introduction

In recent decades, the Mediterranean region has emerged as a climate change hotspot, experiencing a marked increase in the frequency, duration, and intensity of marine heatwaves. These extreme temperature events pose a significant threat to coastal ecosystems and the sustainability of marine aquaculture, particularly for temperature-sensitive species (Martínez et al. 2023). Elevated sea surface temperatures can disrupt metabolic homeostasis, suppress immune function, and increase susceptibility to diseases and parasitic infections in farmed fish. Moreover, prolonged heat stress may reduce feed intake, impair growth performance, and compromise product quality (Stavrakidis-Zachou et al. 2021). As the impacts of climate change intensify, there is an urgent need for adaptive strategies to mitigate thermal stress in aquaculture systems. The inclusion of natural bioactive substances in fish diets has shown encouraging results in enhancing thermal stress resilience. Although studies in this area are still limited, nutritional approaches appear promising in mitigating the impact of climate-induced water warming in aquaculture (Pelusio et al. 2020). In this study, the potential of a dietary supplement, consisting of a blend of organic acids and phytogenic molecules (AviPlus®Aqua) and designed to support gut health, enhance nutrient absorption and improve immune function, to improve growth performance, enhance nutrient absorption and resilience against a high temperature stress, was evaluated in a commercially relevant marine aquaculture species.

## Materials and methods

Groups of n.60 10g European seabass (*Dicentrarchus labrax*) were placed into 6 300 l tanks forming part of a marine water recirculating aquaculture system (RAS). Water quality parameters were recorded daily. Fish were first acclimated on the study diets for one week at 25°C with oxygen content >6 mg/l. After the acclimation period, the thermal stress was started and the water temperature was increased by 1°C/day until the final temperature of 30°C was reached and then maintained for an additional 23 days. The start of the acclimation period was considered as day 0, day 7 is considered the start and day 35 is considered the end of the challenge. Fish were weighed on d0 and d35, and additional sampling was performed on d7 and d35 to evaluate performance, condition indexes and gene expression in target tissues with the focus of investigating intestinal integrity (zonula occludens - *zo-1*, occluding - *occl*, aquaporin - *aqp-1*) and stress response and adaptation (heat shock protein - *hsp-70* and *hsp-90*, glucocorticoid receptor - *gr*). Intestinal samples from each treatment (n = 3 per tanks) were collected and stored in NucleoProtect®. RNA was isolated, quantified, and reverse-transcribed in single-stranded cDNA. Relative gene expression of selected markers was normalized using two housekeeping genes (β-actin and ef1α). The comparative Ct method (2-ΔΔCt method, (Livak e Schmittgen 2001) was used to quantify gene expression. The Shapiro-Wilk and Kolmogorov-Smirnov test were performed for normality and homogeneity check, respectively. Results of the different parameters were analyzed using t-test. All statistical analysis were performed using the software GraphPad Prism v.10.4.1 (GraphPad Software, Inc., Boston, Massachusetts, United States). Differences were considered significant when $p \leq 0.05$, and trends were identified when $0.05 < p \leq 0.1$.

## Results

Statistical analyses (Figure 1) reported enhanced positive performance responses of treated diet compared to the control. Treated diet gave the highest final average weight (+ 5%) and SGR (+ 0.14%/day) while achieving the lowest FCR (-5 points), significantly better than that obtained with fish fed the control, indicative of enhanced feed efficiency. Gene expression analysis revealed modulation of the selected markers, suggesting a potential mode of action of the tested phytogenic blends through their capacity to influence barrier integrity and stress response pathways. In conclusion, these results were achieved within a short trial period, highlighting the high potential of these phytogenic blend in supporting fish performance and general health status under thermal stress.

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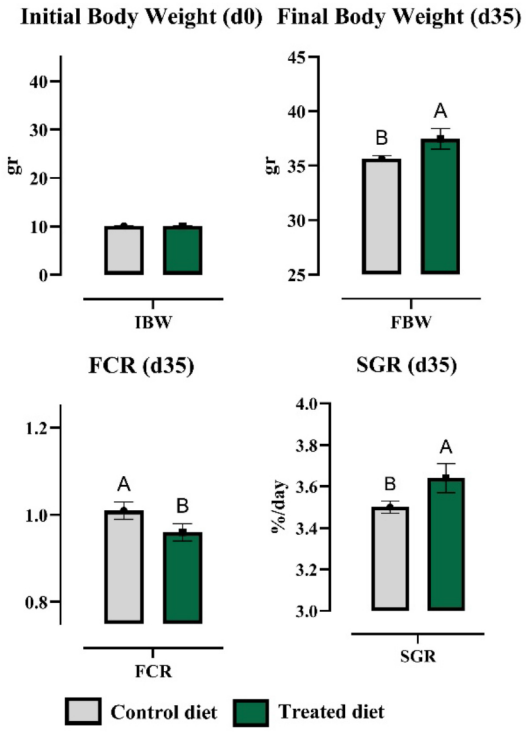


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# COMBINED EFFECTS OF METHIONINE SUPPLEMENTATION AND VACCINATION ON RAINBOW TROUT HEALTH AND DISEASE RESISTANCE AGAINST *Yersinia ruckeri*

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## Introduction

Evidence from fish studies suggests that dietary methionine supplementation can support both innate (Machado et al., 2021, Machado et al., 2018) and adaptive (Martin et al., 2023) immune mechanisms. It is therefore hypothesized that dietary methionine supplementation could be a promising strategy to enhance vaccine efficacy when administered prior vaccination procedures. The present study aimed to evaluate the combined effects of dietary methionine supplementation and vaccination on immune status, antioxidant capacity and response to *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*).

## Material and Methods

Juvenile trout (5.9 ± 0.9 g) were randomly distributed across 32 tanks (n=35) in a semi-open recirculating freshwater system (14 ± 0.5°C) and fed either a control diet (CTRL, 8.0 mg/g DW) or a CTRL-based diet supplemented with methionine (MET, 17.8 mg/g DW). After 4 weeks, half of the fish within each dietary group were either dip-vaccinated (AquaVac® ERM Concentrate, MSD Animal Health) against *Y. ruckeri* or sham-vaccinated to serve as control. At 21 days post-vaccination, samples were collected from two fish per tank (n=16) to assess bacterial load, hematological parameters, differential cell counts, hepatic metabolites, antioxidant defenses and gene expression, head-kidney gene expression and plasma proteomic profiles. The remaining fish were intraperitoneally challenged with *Y. ruckeri* (5 x $10^5$ cfu/ml) or injected with HBSS as a control. The same parameters were evaluated in 9 fish per group at 4, 24 and 48 hours post-infection. Mortality was monitored daily for 13 days. Fish remained on their respective diets throughout the entire trial.

## Results and Discussion

Prior to infection, MET-fed fish showed decreased hepatic *amd1* expression compared to CTRL-fed fish, a difference that persisted following infection. In unvaccinated fish, post-infection analyses revealed that methionine supplementation appeared to compromise antioxidant defenses, evidenced by decreased hepatic SOD activity, GSH levels, and reduced plasma GPx abundance, and to prolong immune activation, indicated by increased expression of *saa* and *cd8b* in the head-kidney and elevated plasma levels of C1q domain-containing protein and chemotaxin. These alterations coincided with the exclusive detection of *Y. ruckeri* in MET-fed fish at 48 hours post-infection and a lower survival rate (75%) compared to CTRL-fed fish (92.5%). In vaccinated fish, no mortality was observed in either dietary group. However, proteomic analyses at 24 hours post-infection revealed distinct responses: MET-vaccinated fish showed increased abundance of hemostasis-related proteins, whereas CTRL-vaccinated fish maintained a proteomic profile more similar to that of pre-infection fish. This difference may have contributed to the observed trend of slightly faster pathogen clearance in the MET vaccinated fish.

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**Acknowledgements**

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# AUTONOMOUS PATHOGEN DETECTION WITH SYNTHETIC BIOSENSORS: PROOF-OF-CONCEPT IN SIMULATED AND NATURAL AQUATIC CONDITIONS

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**Introduction**

Aquaculture has emerged as a sustainable alternative to wild-capture fisheries, offering a scalable solution to meet the growing global demand for protein-rich food. In 2022, aquaculture production exceeded 178 million tons and is projected to grow by 5,5% by 2030[1,2]. However, bacterial diseases, remain a major threat to this industry, accounting for significant mortality and over $6 billion in economic losses annually[3,4]. Traditional diagnostic methods (e.g., culture, PCR, omics technologies) are time-consuming, require specialized equipment, and are impractical for early intervention in field conditions[5,6].

To address this challenge, this study —developed at the start-up KOA Biotech and validated in collaboration with the Universitat Autònoma de Barcelona (UAB)— introduces a novel biosensor-based approach for continuous, point-of-care pathogen detection designed for direct application in fish farms.

**Materials and Methods**

Nine whole-cell biosensors were developed using non-pathogenic E. coli DH5a strains carrying HCN plasmids encoding specific receptors constructs, designed to respond to specific signals. These biosensors were first characterized *in vitro* upon lab conditions to evaluate biosensors' sensitivity, specificity and dynamic response range.

Subsequently, to bridge the gap between laboratory and real-world application, four fish pathogens—*Aeromonas salmonicida*, *Vibrio anguillarum*, *Vibrio harveyi*, and *Photobacterium damselae* subsp. *piscicida*— were cultured at low concentrations in water collected from real fish tanks (in the absence of live fish) under controlled temperature, salinity, and nutrient-depleted conditions. Biosensor responses obtained from these experiments were used to train a machine learning classification model aimed at pathogen identification based on biosensor output patterns.

Finally, validation of the integrated biosensing platform (biosensors and hardware) was performed in collaboration with a specialized aquaculture research facility (e.g., CTAQUA). Trials involved exposure to major aquaculture pathogens (*V. harveyi*, *V. anguillarum*, *A. salmonicida*, and *P. damselae* subsp. *piscicida*) under controlled infection scenarios to evaluate the performance of the system under near-real conditions.

**Results**

Biosensors exhibited strong, concentration-dependent activation in response to their cognate signals, displaying both high sensitivity and distinct activation profiles for each biosensor type, confirming their specificity toward different target molecules.

Under controlled aquaculture-relevant conditions (fish tank water without live fish), biosensors successfully detected early pathogen presence even at low concentrations *in vitro*. Each pathogen induced a distinct biosensor activation signature, enabling the generation of preliminary classification models using machine learning approaches for pathogen identification based solely on biosensor responses.
Preliminary in vivo results further explored pathogen identification with biosensor signals within the first days of an infection, suggesting a strong potential for early diagnosis timely outbreak prevention in aquaculture environments.

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**Conclusions**

This study demonstrates the utility of synthetic biology tools for real-time pathogen detection in aquaculture. The engineered biosensors, integrated with an *in vitro* validation framework and preliminary *in vivo* data, highlight their potential as an effective and scalable point-of-care diagnostic system. The initial *in vitro* setup offers a novel experimental platform to investigate the early virulence stages of pathogenic bacteria and to evaluate the influence of environmental cues on infection processes. This approach enables the development of a high-throughput screening system that circumvents the logistical, ethical, and financial constraints associated with *in vivo* fish trials, while still generating robust and scalable data.

Overall, the early pathogen detection technology developed at KOA Biotech represents a promising step toward sustainable aquaculture management, aligning with FAO objectives[7] to reduce antibiotic dependence and enabling timely, preventive interventions that can mitigate disease outbreaks and improve fish health and farm productivity.

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# *ACETYLCHOLINE* CONTROL OF METAMORPHOSIS IN *Patella aspera*

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**Introduction**

The full-cycle aquaculture of marine invertebrates requires the metamorphosis induction of the swimming larvae into benthic post-larvae for the grow-out phase. Recent advances in limpet aquaculture used coralline algae and conditioned seawater as natural inducers (Castejón et al. 2024). Compounds with settlement inducing properties, such as acetylcholine, are easier to store and manage than natural inducers and can be applied on demand. The present research determined the influence of acetylcholine on the metamorphosis of the limpet *Patella aspera*.

**Material and Methods**

The broodstock was captured between February and March 2024 and kept in an open system (20 ± 1 °C and 36 ± 1 psu). Gametes were obtained by dissection. Then, the oocytes were matured artificially ($NH_4OH$ at pH 9 during 20–30 min.) and fertilized *in vitro* ($10^5$ sperm cells $ml^{-1}$). Lecithotrophic larvae were cultured in glass beakers and plastic jars (16 °C).

Two assays placed pediveliger larvae 3-day old in culture wells (8 mL) and used two control treatments, filtered seawater as negative control and coralline algae as positive control. First assay tested four acetylcholine (ACH) concentrations (2 to $5\times10^{-3}$ M). Second assay tested $4\times10^{-3}$ M ACH alone and a combination of $4\times10^{-3}$ M ACH with coralline algae. Treatments were applied on larvae 7 and 10-day old, respectively. Assays were finished four days later. The third assay was designed to test the impact of ACH on the completion of metamorphosis. For this purpose, metamorphics (settled specimens that just lost their velum) were obtained by exposing 10-day old larvae to coralline algae for 24h. Then, the metamorphics were exposed to filtered seawater (negative control) or $4\times10^{-3}$ M ACH for three additional days.

Two settled stages were identified: metamorphics (velum loss) and post-larvae (teleoconch and head development). The percentage of settlers (metamorphics + post-larvae) determined the success in starting the metamorphosis and the percentage of post-larvae determined the success in finishing the metamorphosis.

**Results and Discussion**

In Assay 1, the percentage of settlers increased in parallel with the concentration of acetylcholine (Fig. 1a), but no post-larvae were observed in acetylcholine treatments (Fig. 1b).

In Assay 2, the coralline algae alone resulted in a similar percentage of settlers as coralline algae combined with acetylcholine (Fig. 1c), but in this combined treatment the percentage of post-larvae was significantly reduced (Fig. 1d). The third assay confirmed the impact of acetylcholine on the completion of metamorphosis, as the percentage of settled specimens (metamorphics) reaching the post-larval stage (head and teleoconch development) was lower (t-test $P < 0.01$) when exposed to acetylcholine (69 ± 13%) than to filtered seawater (90 ± 7%).

Acetylcholinesterase is a neuronal enzyme found in both vertebrates and invertebrates involved in the hydrolysis of the neurotransmitter acetylcholine. Jiao et al. (2019) identified a high diversity of nicotinic acetylcholine receptor genes in molluscs, suggesting a high diversity of functions, including a likely regulation of early development. The present results supported this hypothesis, as acetylcholine promoted the start of metamorphosis in limpets (velum loss), while inhibiting the completion of metamorphosis. Since in the marine gastropod *Concholepas concholepas* the metamorphosis entails a new form of acetylcholinesterase (Inestrosa et al. 1993), it is congruent to postulate that the regulation of metamorphosis in marine gastropods may require the presence and posterior reduction of acetylcholine levels.

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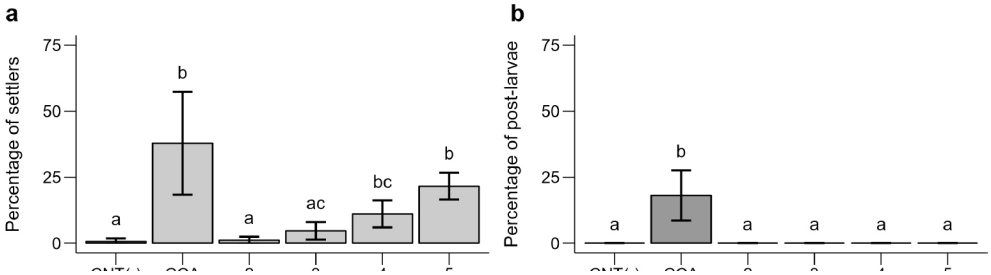


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# LONG-TERM EFFECT OF DIETARY SUPPLEMENTATION WITH ALGAE ON JUVENILE TURBOT (*Scophthalmus maximus)* GUT MICROBIOME

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## Introduction

As the aquaculture sector continues to grow at a rapid rate, it is necessary to explore effective nutritional supplements to improve the growth and welfare of the fish. Macroalgae and microalgae have been widely evaluated in the recent years as a novel alternative protein resource for aquafeeds (Gu et al., 2021), and in this regard, algae have been recognized as having promising potential relating to their nutritional value, and sustainability in production. Nevertheless, diets based on vegetable sources continue to present significant challenges for carnivorous fish, as these formulations may limit the efficiency of nutrient digestion and assimilation, and, in certain instances, are associated to the onset of intestinal inflammatory responses (Colombo, 2020). Diet plays an important role in causing intestinal dysbiosis, leading to disruptions to the normal microbial balance. The objetive of this study is to assess the impact of a algae-supplemented diet on microbiota composition of juvenile turbot (*Scophthalmus maximus*) for a period of 180 days.

## Materials and methods

Turbot specimens used in the present study were moved to Marine Aquaculture Plant of Santander (MAPS) (IEO-CSIC, Santander, Spain), where the experiment was conducted. A 6-month start feeding trial was conducted by feeding tanks of turbot. Triplicate groups were fed with 4 differents diet: i) control comercial diet (CTC); ii) A new fish meal diet was formulated (Saéz et al., 2022) and regarded as a control diet (CT), according to the nutritional requirements for turbot; iii) the control diet with a blend of freshwater and marine microalgae supplemented (M5), and iv) the control diet with 5% macroalgae supplemented (*Ulva rigida.*).

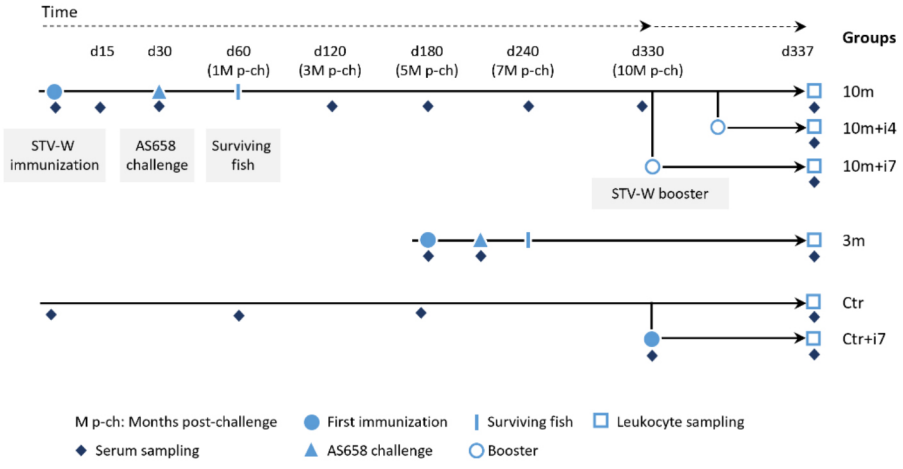


Fig.1. Schematic representation of the immunization schedule for experimental groups to analyze long-term immune responses. Fish were immunized using different protocols (see legend).

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A total of 72 intestinal mucus samples were subjected to DNA extraction using DNeasy Blood & Tissue kit (Qiagen) and DNA purity and concentration were obtained. DNA metabarcoding library preparation and sequencing were carried out by AllGenetics & Biology SL (www.allgenetics.eu). Sequences were quality filtered and taxonomically assigned following a pipeline using SILVA database. The bioinformatic data processing involved a workflow based on the DADA2 library in Qiime2, and different package of R to analyse taxonomic composition and assess microbial diversity.

**Results and discusión**

The main objective of the study was to assess how the intestinal microbiota of fish responds to feeding with algae-based diets. The preliminary results show that the juvenile turbot accepted the alternate feeding (CT, M5 and U5) diets with no negative effects on survival or growth performance. No differences were observed independently of the diet with respect to diversity alpha (Shannon-index).

In relation to microbiota composition, the phylum with the highest representation was proteobacteria, followed by Firmicutes and Bacteroidota (Fig. 1). M5 and U5 reported an increase of Firmicutes and decrease of Bacteroidota presence compare to the control diets (CT and CTC).

The results suggest that diets M5 and U5 promote a differential shift in the intestinal microbiota of turbot toward the growth of bacteria from the phylum Firmicutes. Based on the results obtained in the microbiota study, the two algae-based diet could be used as additives for aquaculture diet supplementation, although further research is required.

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**Acknowledgments**

This work was supported by ALGADIET II project developed with the collaboration of the Biodiversity Foundation of the Ministry for Ecological Transition and the Demographic Challenge and financed by 2019 Pleamar Programme and the European Maritime and Fisheries Fund.

# MONOCLONAL ANTIBODIES AGAINST SOLE IGM GIVE CLUES ON THE SECONDARY IMMUNE RESPONSE IN FLATFISH

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## Introduction

Upon activation, B cells undergo a differentiation process, first becoming plasmablasts and eventually maturing into plasma cells, which possess an enhanced capacity to secrete antigen-specific antibodies (1). These antibodies are a critical component of the specific immune system in fish and play a central role in vaccine induced protection (2). However, a comprehensive understanding of how cell-mediated immunity is orchestrated in fish- including the dynamics of immune response transitions and the mechanisms underlying the generation and persistence of immunological memory-remains incomplete (3, 4). *Solea senegalensis* is a commercially important species in aquaculture, and the development of vaccines is of great interest. Currently, autogenous vaccines offer partial protection against furunculosis, but their efficacy is limited (5). In this study, monoclonal antibodies (mAbs) specific for sole IgM were generated, which allowed us to evaluate the kinetics of $IgM^+$ B cell responses, assess the recruitment of these cells to the peritoneum following vaccination, and compare the dynamics of antigen-specific antibody-secreting cells (ASCs) in the spleen and kidney of primed and boosted fish.

## Material and methods

We evaluated the efficacy of two vaccines formulated with whole (STV-W) or sonicated (STV-P) bacterin from an atypical *Aeromonas salmonicida* strain and the dynamics of the immune responses. All the experiments described in animals were carried out in accordance with the Guidelines of the European Union Council (2010/63/EU). Generation of hybridomas was performed based on the method of Galfre and Milstein (6). The experimental plan for fish vaccination and sampling points for both immunized and control groups are detailed in Fig. 1.

The efficacy of the vaccines was analyzed by challenging sole at 30-, and 90-days post-vaccination by ip. inoculation with a lethal dose that was previously determined in a LD50. The relative percent survival was calculated using the formula: RPS = [1 – (% mortality in vaccinated fish/% mortality in control fish] x 100%. For leukocyte analysis, tissue and peritoneal single cell suspensions (40 µm pore size nylon mesh) were centrifuged onto a 30/51% discontinuous Percoll gradient. Labelled cells were analyzed in a CytoFlex flow cytometer and sorted in a BD FACSAria III. Real-time PCR was performed in a QuantStudioTM 3 System (Applied Biosystems).

## Results

Both vaccines conferred significant protection compared to the non-vaccinated control at 1 month after immunization, which included activation of pro-inflammatory genes, cell mobilization to the injection site, and generation of specific antibodies. The formulation containing whole bacteria (STV-W) showed a better RPS, 48% compared to 34% for the particulated bacteria. However, when the challenge was performed at 3 months post-vaccination, no protection was observed with either vaccine, indicating that these formulations provided short-term protection. To further investigate the response, we have generated two mAbs against sole IgM (CETGA-110 and CETGA-111) for use in various immunoassays, including ELISA, WB, immunofluorescence, and flow cytometry. Immunofluorescence images and flow cytometric analysis of cells labelled with

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these mAbs revealed a characteristic B cell profile, consistent with B lymphocyte descriptions from other fish species (7). Moreover, the percentages of total sole $IgM^+$ cells in spleen (~30%) and kidney (~7%) isolated leukocytes are comparable to those described for other fish species (8), and FACS-sorted positive populations from these tissues expressed large amounts of both secreted and total IgM. Interestingly, a booster administered ten months post-challenge to previously vaccinated and surviving fish led to activation of ASCs analyzed by ELISPOT, and a more rapid production of specific IgM antibodies compared to primed fish.

**Conclusions**

- Standard autogenous vaccines formulated with the bacterin of *A. salmonicida* subsp. *achromogenes* provide moderate short-term protection in sole, including activation of pro-inflammatory genes, cell mobilization to the injection site, and generation of specific antibodies. However, this protection does not persist beyond three months without a booster.

- A booster administered to vaccinated and surviving fish ten months after the challenge led to an activation of antigen-specific ASCs, and specific antibodies were produced earlier in these fish than in those receiving the vaccine for the first time, indicating that sole is capable of mounting a secondary immune response upon re-exposure.

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# WHITE GRAPE MARC EXTRACTS AS POTENTIAL MODULATORS OF THE OXIDATIVE STRESS STATUS OF EUROPEAN SEABASS

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**Introduction**

Aquaculture is a rapidly expanding sector that is essential to global food security, with fish and seafood consumption projected to increase by 27% by 2030. However, intensive production systems often expose fish to environmental stressors such as temperature fluctuations, hypoxia, salinity changes, and overcrowding that can induce oxidative stress. Reactive oxygen species (ROS) play important roles in the functioning of the immune system, namely in activating various cellular signalling pathways. On the other hand, an excess of ROS can damage cells and trigger cell death. Additionally, potential bacterial infections further exacerbate ROS overproduction.

Grape marc, a residue from the wine industry, is a rich source of antioxidants, due to its high concentration of polyphenols. The antioxidant and antimicrobial potential of grape marc extracts is being explored within the NeoGiANT project that aims to develop and validate innovative natural formulations based on white grape polyphenols for animal production, including aquaculture. In this work, we assessed the potential of three white grape marc extracts, AquaGrapes (obtained with an alcohol, A, a glycol, G, and a ketone, K), to act as modulators of the European seabass oxidative status, by evaluating target oxidative stress markers.

**Material and methods**

Five isoproteic (46%) and isolipidic (19%) diets were formulated to meet the nutritional requirements of European seabass (*Dicentrarchus labrax*) juveniles, including a control and four experimental diets incorporating different formulations of AquaGrapes (alcohol extract at 450 ppm – A450, glycol at 450 ppm – G450 and ketone extract at 450 ppm and 1500 ppm, K450 and K1500 respectively). Diets were prepared using finely ground ingredients, with the AquaGrapes additive added in liquid form during mixing, followed by pelleting (3 mm), drying (60 °C, 48 h), and storage at −20 °C until used. A 48-day feeding trial was conducted in a thermo-regulated recirculating system (15 tanks, 90 L each; 23.8±0.47 °C; 31.7±1.34 ppm salinity; 7.6±0.4 mg/L $O_2$; 12L:12D photoperiod). Juvenile seabass (45 g) were randomly distributed into 15 groups (3 replicates per diet, 15 fish/tank) and fed twice daily until satiety. At the end of the trial, three fish per tank were euthanised and sampled 4 hours post-feeding. The liver was collected and immediately frozen in liquid nitrogen. Oxidative stress enzymes analysis was performed as described in Magalhães *et al.* (2022).

**Results**

Fish fed the G450 diet presented higher levels of catalase (CAT) ($p$=0.016) activity and lower lipid peroxidation (LPO) when compared to the control diet. Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Glutathione reductase (GR) activities for the diet G450 were higher than the control diet but not statistically different ($p$=0.064, 0.771, 0.778, respectively). Diets A450, K450 and K1500 presented higher absolute values of SOD activity than the control diet, even though these weren't statistically different.

**Conclusion**

AquaGrapes additives, particularly the G extract included at 450 ppm, are promising positive modulators of the oxidative status in aquaculture fish.

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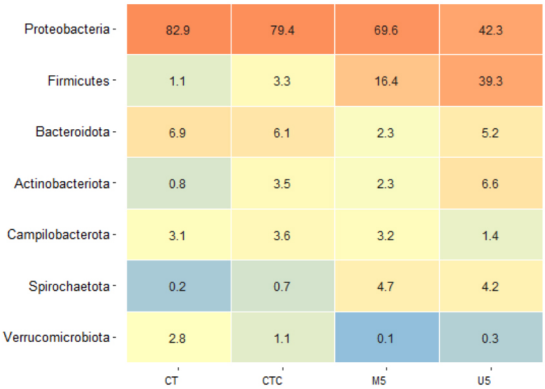


Figure 1. Heatmap generated using the relative abundance of bacteria al phyla_class level.

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# THEORETICAL INTEGRATION OF ESG METRICS AND SDG TARGETS FOR BIOMASS AND BIOACTIVE COMPOUND OPTIMIZATION IN MEDITERRANEAN AQUACULTURE SYSTEMS

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As global aquaculture confronts dual imperatives of food security and ecological sustainability, innovative frameworks are urgently needed to reconcile biomass productivity with environmental and social equity. This study presents a novel theoretical model for Mediterranean aquaculture that embeds Environmental, Social, and Governance (ESG) principles and United Nations Sustainable Development Goals (SDGs) into biomass and target compound optimization strategies. Addressing critical gaps in current sustainability paradigms, we propose a circular bioeconomy approach that leverages Mediterranean biodiversity to maximize resource efficiency while preserving coastal ecosystem integrity.

At its core, the framework introduces dynamic algorithms to balance species-specific biomass yields (finfish, mollusks, algae) with the extraction of high-value compounds (nutraceuticals, antioxidants), guided by SDG 2 (Zero Hunger), SDG 12 (Responsible Production), and SDG 14 (Marine Ecosystems). We conceptualize three interconnected pillars:

1. Multi-Trophic Synergy – Spatial modeling of integrated aquaculture systems to minimize waste and amplify compound recovery.
2. Climate-Adaptive Governance – AI-driven biomass forecasting tools to address Mediterranean warming and habitat vulnerability (SDG 13).
3. Social License Innovation – Metrics linking equitable value-chain participation (SDG 8, 10) to ecosystem service valuation.

Theoretical scenarios demonstrate how precision aquaculture—combining life cycle assessments with ESG-aligned policies—could reduce feed conversion ratios by 15–20% while enhancing bioactive compound yields. By prioritizing endemic species (sea bream, microalgae), the model unlocks niche markets for marine-derived pharmaceuticals, contingent on regenerative practices that protect biodiversity hotspots.

This work challenges traditional productivity metrics, advocating instead for SDG-linked KPIs that quantify carbon sequestration, coastal employment, and nutrient recycling. We further argue that Mediterranean nations, as climate change frontlines, require EU-funded R&D partnerships to operationalize this framework—aligning with AE2025's vision of transdisciplinary innovation.

# TOWARDS ANNOTATION-FREE FISH MONITORING IN AQUATIC ENVIRONMENT BASED ON VISION TRANSFORMERS

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## Introduction

Monitoring fish behavior in aquaculture environments is essential for welfare assessment, early detection of anomalies, and production efficiency. However, existing computer vision methods for fish monitoring tasks often rely on large annotated datasets, which are scarce in underwater context due to the time-intensive effort of manual labeling.

This work presents a fully unsupervised pipeline for instance-level fish segmentation and skeletal pose estimation. These pose representations enrich the object-level information beyond the spatial information provided by the bounding boxes in object detection, and also support robust individual tracking and individual behavioral analysis.

Unlike fish segmentation methods based on static background, this approach works under moving camera conditions. Thus, this work contributes to scalable, annotation-free monitoring systems in precision aquaculture.

## Materials and methods

In this work, it is proposed a methodology based on a modular pipeline, illustrated in Figure 1, which comprises three stages designed to transform a video frame into a skeletal pose estimation of individual fish based on keypoints head, body, and tail.

The first stage uses Grounding DINO (Liu et al, 2024), an extension of DINOv2, which learns visual features without labeled data. It integrates language models to associate textual prompts (e.g., ”fish”) with image regions, enabling multi-instance object segmentation without task-specific training.

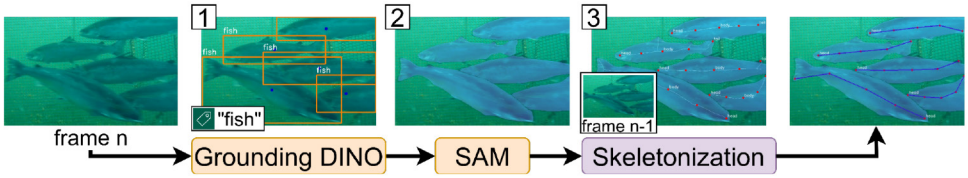


Figure 1: Block-diagram representing method stages pipeline.

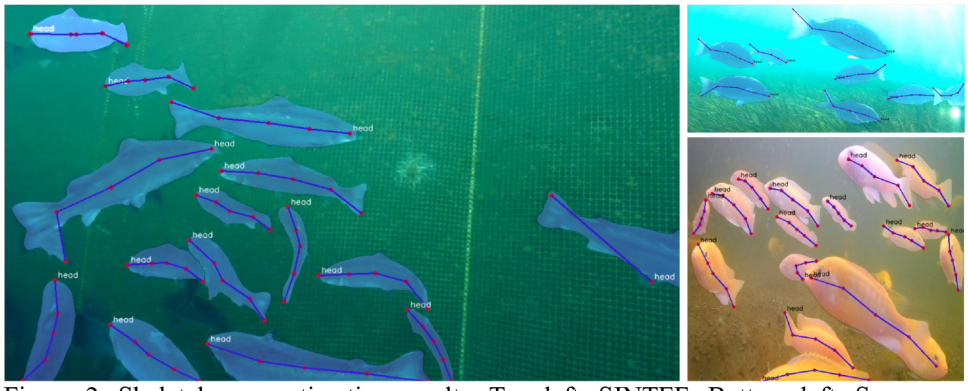

Figure 2: Skeletal pose estimation results. Top left: SINTEF. Bottom left: Seagrass. Right: *Etro plus maculatus*

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Once fishes are detected, the second stage employs the pre-trained *sam vit h* variant of the Segment Anything Model (SAM) (Kirillov et al., 2023) for instance segmentation, generating accurate binary masks for each fish detected by DINO using the bounding boxes and the centroid points as prompts, without requiring specific domain annotations.

Finally, the third stage involves skeletonizing the binary mask to create a one-pixel-thick representation of the fish, which is then treated as a graph. The longest path in the graph defines the main axis, with keypoints for the head and tail at its endpoints. Optical flow is subsequently applied to refine head-tail assignment based on movement between frames. The body keypoint is located near the centroid of the fish, and two midpoints along the head-body and tail-body segments are computed to more accurately represent its shape. These five keypoints form a reliable basis for tracking and analyzing the shape over time.

This skeletal pose estimation goes beyond traditional object detection and tracking methods, which typically rely on bounding boxes, by enabling the analysis of fish orientation, social interactions, and behavioral responses to environmental stimuli through keypoint based identification.

**Results**

The proposed unsupervised pipeline was evaluated on three publicly available underwater video datasets representing diverse aquatic scenarios. The main evaluation was conducted on the OLAQUA SINTEF dataset, which contains real-world deployment footage from large-scale aquaculture offshore facilities, characterized by camera movements, dynamic backgrounds, high-density fish population, and low image quality conditions.

Additional qualitative assessment was performed on two additional datasets: one with nonconsecutive frames of *Etroplus maculatus*, and Seagrass (Ditria et al.), both recorded in the wild with a fixed camera. Although the latter dataset includes ground truth annotations, only qualitative results are reported for consistency (see Figure 2). Visual inspection of all these datasets confirms the generalization of the pipeline across environments, species, and recording conditions without requiring annotated training data for computer vision deep learning models.

In conclusion, this work presents a novel, fully unsupervised pipeline for fish instance segmentation and skeletal pose estimation in non-fixed underwater camera videos, using recent vision transformer models (DINOv2 + SAM), followed by a skeletonization module. This approach eliminates the need for manual annotations while producing rich spatio-temporal descriptors of fish posture (head-body-tail) that enable fine-grained behavioral insights.

By reducing the annotation burden and enabling pose-aware fish monitoring, this work contributes to scalable, annotation-free monitoring in precision aquaculture.

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# REDUCING THE CARBON FOOTPRINT OF RAINBOW TROUT (*Oncorhynchus mykiss*) FARMING: EVALUATION OF BARLEY PROTEIN CONCENTRATE AS A SUSTAINABLE FEED INGREDIENT

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**Introduction**

The rainbow trout (*Oncorhynchus mykiss*) is one of the most widely farmed freshwater fish species globally, known for its rapid growth, high feed efficiency, and valuable nutritional profile. Its adaptability to various farming conditions makes it an ideal species for evaluating alternative and sustainable feed ingredients. One such ingredient is barley *(Hordeum vulgare* L.), which is widely used as a raw material in the starch industry and generates protein-rich by-products that can be valorised in both the food and feed sectors (Yalcin et al., 2007). In feed production, barley is considered an excellent source of nutrition and energy. Additionally, it plays an important agronomic role by contributing to nitrogen accumulation in the soil, improving soil fertility, and reducing the need for nitrogen-based fertilizers. Moreover, barley's ability to stimulate soil microbial activity makes it a valuable precursor crop in sustainable farming systems (Lammas and Shitikova, 2024).

Beyond its agronomic value, barley also offers substantial environmental benefits, particularly in terms of reducing the carbon footprint of aquafeeds. Preliminary evaluations conducted within the framework of the present study indicate that replacing fishmeal with increasing levels of Barley Protein Concentrate (BPC) can lower greenhouse gas emissions associated with feed production: from 4887.3 kg $CO_2$ eq./ton in the control diet to 4133.5 kg $CO_2$ eq./ton in the diet containing 30% BPC, representing a 15.4% reduction. These findings reinforce the potential of BPC as a climate-friendly alternative ingredient.

In this study, we specifically assessed the effects of increasing dietary inclusions of BPC on growth performance, digestive enzyme activities, intestinal and hepatic histology, and fillet quality traits in rainbow trout.

**Materials and Methods**

Three experimental diets containing increasing levels (10%, 20%, and 30%) of BPC and a corresponding progressive reduction in fish meal content (5%, 2.5% and 0%) were tested. A fourth diet, without the inclusion of BPC, was used as a control. Additionally, astaxanthin was included in all experimental diets to allow flesh pigmentation (salmon coloration) in the trout. Diets were tested on triplicate fish groups of 50 individuals (initial weight: 123 g) over a period of 90 days. During the trial, feed was provided to apparent satiation, temperature was kept at 15.0 ± 1.0 °C and oxygen above 10.5 mg $L^{-1}$. At the end of the trial, final body weight (FBW), specific growth rate (SGR), feed intake (FI) and feed conversion rate (FCR) were calculated. Total alkaline protease, trypsin, chymotrypsin, pepsin, α-amylase, and bile salt-activated lipase were tested to evaluate the enzymatic activity. Histological samples of liver and intestine were taken to evaluate the tissue response. Samples of the fillet were taken to perform instrumental analyses and a sensory panel test to investigate potential organoleptic differences between the diets.

Differences among treatments is considered significant at $p < 0.05$.

**Results and Discussion**

Fish fed diets with up to 20% BPC showed growth performance, feed intake, and feed conversion ratio comparable to the control, consistent with previous findings in salmonids fed with BPC (Bell 2016). At the highest inclusion (30%), slight reductions in growth and nutrient efficiency indices occurred, due to reduced protein digestibility and fiber content.

Digestive enzyme activities were moderately influenced by dietary BPC. Literature on trout suggests proteases and lipases activity can decrease slightly at lower inclusion levels of fishmeal and higher inclusion of BPC, due to anti-nutritional factors (Zaretabar 2021). Nonetheless, enzyme activities remained within physiological ranges, suggesting preserved digestive function.

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Histological examination of intestinal tissue revealed well-preserved mucosal structure in fish fed up with 20% BPC, with normal villi height and enterocyte integrity. At 30%, minor enteritis or mucosal thinning appeared, as noted in similar studies with high inclusion of plant proteins (Romarheim 2008). Hepatic histology remained within normal parameters, though slight hepatocyte vacuolization or lipid accumulation was observed at the highest BPC level, possibly indicating early metabolic adaptation. Texture and color parameters showed minor variation across diets. Sensory evaluation confirmed acceptable texture and flavor across all groups, though minor differences in some organoleptic properties of flesh were observed, particularly at higher BPC inclusion levels, as noted by de Francesco (2008) in a study with inclusion of a plant protein mixture.

**Conclusion**

Overall, the trial confirmed that moderate levels (10–20%) of BPC can replace fishmeal without adverse effects on growth, health, or fillet quality in rainbow trout, supporting its application in sustainable aquafeeds. Given the global significance of rainbow trout in freshwater aquaculture, it is essential to investigate feed ingredients that can contribute to reducing the carbon footprint of its farming.

**Acknowledgements**

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# DETERMINATION OF THE WEIGHT OF CUTTLEFISH (*Sepia officinalis*) HATCHLINGS AND JUVENILES WITHOUT MANIPULATION BY COMBINED IMAGE AND REGRESSION ANALYSIS

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## Introduction

Cephalopods have a soft, elastic and delicate skin, being especially sensitive to handling. In this regard, some authors have reported mortality in cuttlefish (*Sepia officinalis*) during samplings, behaviour changes, small wounds from rubbing against tank walls, and escape and inking responses during cleaning or capture tasks (Forsythe et al., 2002). Skin lesions are also associated with various types of bacterial infections (Farto et al., 2019). In any case, it is sometimes necessary to capture animals to obtain biometric measurements and verify the suitability of a diet or health status. To avoid the risks associated with handling, the objective of this study was to develop a system for monitoring the weight and length of cuttlefish hatchlings and juveniles without removing them from the water.

## Material and methods

Cuttlefish (*S. officinalis*) hatchlings were obtained from wild breeders kept at the IMIDA marine aquaculture station (San Pedro del Pinatar, Murcia). Subsequently, hatchlings were moved at 15 100-L tanks (30 for each tank; stocking density150/$m^2$) and held in a temperature-controlled recirculating seawater system. The animals were fed live adult artemia or mysids for 30 days. Two rectangular pieces of laminated graph paper (14x9 cm) were glued to the bottom of the tanks (Fig. 1A). This study consisted of two phases: 1) obtaining biometric data for the development of equations and 2) applying the method. 1) In 9 tanks, the animals were captured, weighed, and measured at 0, 15, and 30 days of life. Photographs were taken for image analysis using ImageJ (NIH, USA), obtaining the following measurements: body weight (BW, g), total length (TL, mm), dorsal mantle length (ML, mm), body width (WI, mm), and area (A, $cm^2$). Both live healthy animals and those died from unknown causes were included. The data were fitted using regression analysis to a potential curve type lnBW = lna+blnX, where "X" represents the independent variable (TL, ML, WI or A). The best equation was selected based on the highest coefficient of determination ($R^2$) and the lowest estimation error (SEE). The statistical analysis was performed using SPSS Statistics 25.

2) In 6 tanks, overhead photographs were used to obtain biometric data and estimate the BW of cuttlefish according to the equations previously developed without removing the animals from the water. Hatchlings were fed artemia or mysids (3 tanks per diet) for 30 days. From day 30, the juveniles were transferred to 2500 L tanks and fed live shrimp (*Palaemon varians*). Additional measurements were obtained on days 40 and 52 to expand the prediction interval of the model.

## Results

The observed ranges for BW, TL, ML, WI, and A were 0.07–2.48 g, 0.94–3.47 mm, 0.50–2.47 mm, 0.49–1.88 mm, and 0.41–4.79 $cm^2$, respectively. All data fitted the proposed equations to a statistically significant extent ($P<0.001$; Table 1); however, the best variable for body weight estimation was cuttlefish area, which explained 96% of the variance of the data with the lowest error in BW estimation (0.157). Secondly, the ML explained more than 92 % of the variance and showed an error of 0.221. The worst predictors for BW were TL and WI. The images and equations allowed monitoring the biometry of animals throughout trials avoiding manipulation. In this respect, higher estimated body weight was showed in cuttlefish hatchling fed mysids (0.78±0.09 g) compared with artemia (0.51±0.10 g) on day 30 (Fig. 1B).

## Discussion and conclusion

Studies that carry out frequent sampling, even every 5, 7 or 10 days, to determine cuttlefish growth are common (e.g., Sykes et al., 2014). Generally, weight or dorsal mantle length are obtained, since total length or width can be influenced by the animals' elasticity or arm position. In fact, according to our results, higher error was obtained in the estimation of BW as a function of TL or WI. The present study offers an alternative for determining cuttlefish biometry during the early stages of life without manipulating them and contributing to the welfare of this cephalopod during rearing trials.

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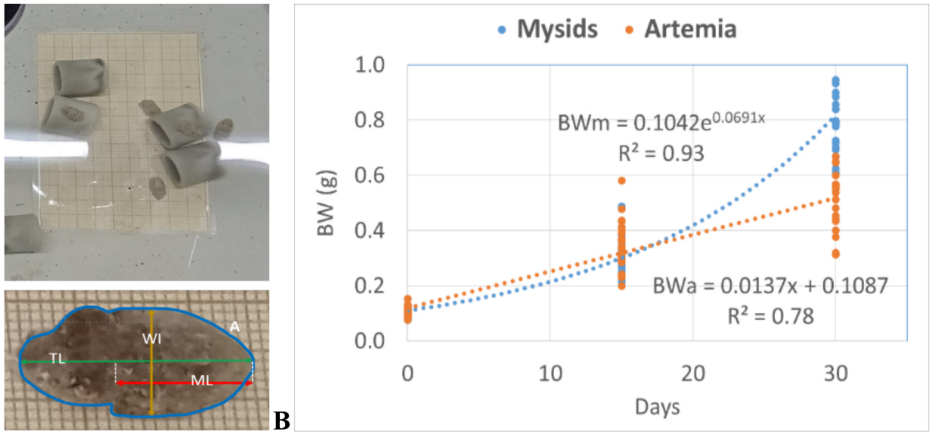
**Table 1**. Results of regression analysis for body weight (BW) as a function of total length (TL), dorsal mantle length (ML), width (WI) and area (A).

| Variable | n | a±SE | b±SE | $R^2$ | SEE | ANOVA |
|---|---|---|---|---|---|---|
| TL | 127 | 0.075±0.004(*) | 2.758±0.075(*) | 0.92 | 0.265 | (*) |
| ML | 127 | 0.284±0.006(*) | 2.537±0.057(*) | 0.94 | 0.221 | (*) |
| WI | 127 | 0.563±0.014(*) | 2.675±0.082(*) | 0.90 | 0.248 | (*) |
| A | 127 | 0.274±0.005(*) | 1.425±0.027(*) | 0.96 | 0.157 | (*) |

lnBW = lna+blnX. where X = TL, ML, WI, or A. (*)P<0.001
a, b: Coefficients of the equations; SE = Standard error; SEE = Standard error of estimate.




**A** **B**

**Fig. 1 (A-B)**. Obtaining biometric measurements (A) and estimated body weight (B) in *Sepia officinalis* fed artemia (BWa) or mysids (BWm) avoiding their manipulation.

**Acknowledgements**

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# SUITABLE DISSOLVED OXYGEN LEVELS FOR SEA BASS (*Dicentrarchus labrax*) MAINTENANCE AT DIFFERENT TEMPERATURES

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## Introduction

Sea bass (*Dicentrachus labrax*) is currently the most produced fish in Spain, ranking third in the EU after rainbow trout and sea bream (Apromar, 2024). Its production is carried out in offshore cages, where stocking densities and climatic conditions are highly variable. In this regard, the high temperatures (>28°C) recorded during the summer for more than a decade in the western Mediterranean (https://portus.puertos.es/#/) are remarkable, which can negatively affect fish growth and health. Moreover, these high temperatures increase oxygen requirements, decrease oxygen solubility and promote algal growth in nets, affecting water exchange. In fact, oxygen supply in sea cages has been considered as a limiting factor in stocking density (Besson et al., 2016). The objective of this study was to determine suitable oxygen levels for sea bass at different temperatures according to the response of ventilation frequency and oxygen consumption to progressive hypoxia.

## Material and methods

Sea bass (*D. labrax*) weighing 145±5 g were used, performing two assays at temperatures of 20.4±0.1 and 26.6±0.7°C. In each assay, three replicates of six fish were place in separate 160 L aquaria-respirometers. When the fish were completely acclimated and showed a regular daily feeding rate (2 weeks), they were subjected to a 24-hour fast before measurements. Oxygen consumption ($MO_2$ in $mgO_2$/kg/h) was determined simultaneously in the three replicates from the drop in oxygen concentration at 30-minute intervals after the water supply was stopped. For this purpose, an oxygen sensor (Hatch LDO101), a filter to remove excretion products, a small pump to homogenize the water, and a floating plastic film on the surface to prevent the exchange of oxygen with the air were placed in each aquarium. Ventilatory frequency (Vf) in beats/minute (bts/min) was visually counted in 5 randomly selected fish at 30-minute intervals. The following data were obtained from each replicate: initial Vf at 100 % oxygen saturation (Vfini), maximum Vf reached by a fish (Vfmax), oxygen saturation (% Sat.) where Vf is significantly modified (Ovf), and critical oxygen concentration (Oc) where oxygen consumption decreases significantly ($P<0.05$). Oxygen levels were restored after reaching Oc.

## Results

Initial ventilatory frequency at normoxia (100% oxygen saturation) ranged from 65±3 to 80±6 bts/min, increasing significantly with temperature ($P<0.05$). Subsequently, sea bass responded to the decrease in oxygen concentration by significantly increasing ventilation frequency from 60.7±2.3 % Sat. at 20.4°C and 71.7±6.4 % Sat. at 26.6°C ($P<0.05$). Sea bass behaved as a regulatory organism (Fig. 1), maintaining constant oxygen consumption up to critical values of 44.7±6.4 % Sat. (3.24 $mgO_2$/l) and 65.2±12.9% Sat. (4.24 $mgO_2$/l) at temperatures of 20.4 and 26.6°C, respectively ($P=0.07$). There were no significant differences between Vfmax reached at 20.4ºC (108±1 bts/min) and 26.6ºC (118±9 bts/min; $P>0.05$).

## Discussion and conclusion

In the range of fish weight and temperatures tested, our findings suggest an optimal dissolved oxygen saturation of above 61 % Sat. at 20.4ºC (4.42 $mgO_2$/l) and 72 % Sat. at 26.6ºC (4.68 $mgO_2$/L) where Vf and $MO_2$ remain constant. Below Ovf, circulatory and ventilatory mechanisms would be activated to cover oxygen requirements, but these are energetically costly and negatively affect growth. Values below 45% Sat. at 20.4°C and 65% Sat. at 26.6°C would be dangerous (altered Vf and $MO_2$), activating anaerobic metabolism and lactic acid production. According to Montgomery et al. (2019), Oc values were even lower at 18°C (23.4% Sat; 131 g).

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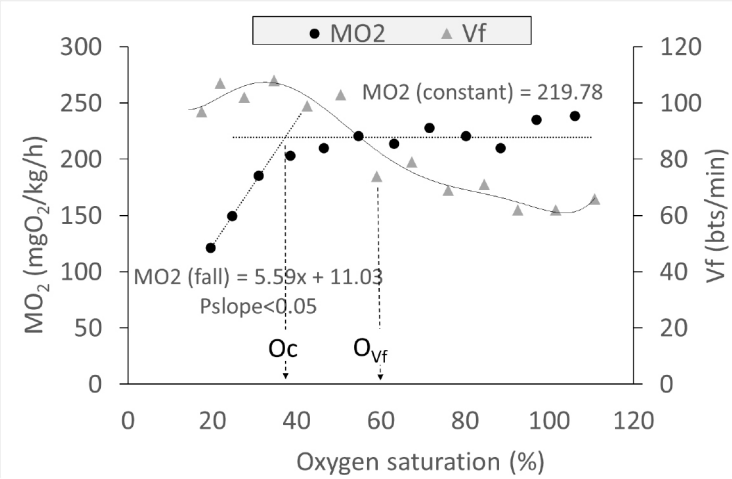


**Fig 1**. Oxygen consumption ($MO_2$) and ventilatory frequency (Vf) as a function of oxygen saturation in sea bass (147 g; 20,4ºC). Critical oxygen saturation (Oc) and the point at which oxygen saturation modifies Vf (Ovf) are marked.

As in other fish species, Ovf and Oc values increase with temperature, indicating that oxygen levels must be higher to cover their metabolic requirements. Ovf values in sea bass were similar compared with *Sparus aurata* (66.8% Sat at 20.5°C and 560 g; Cocco et al., 2009) or *Dentex dentex* (71.4% Sat at 28.1°C and 251 g; Cerezo Valverde et al., 2006). However, the higher Oc values in sea bass at high temperatures (65% Sat at 26.6°C) compared to other species such as *D. dentex* (40.2% Sat at 28.1°C; Cerezo Valverde et al., 2006) were remarkable. These results reinforce the importance of maintaining high oxygen levels (>75-80% Sat.) especially in sea bass during the warmer months due to its greater sensitivity to temperature respect to other species of interest in aquaculture.

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# CRITICAL OXYGEN LEVELS AND VENTILATORY FREQUENCY IN GOLDEN GREY MULLET JUVENILES (*Chelon auratus*) AT HIGH TEMPERATURE

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## Introduction

Grey mullets are cosmopolitan fish that can tolerate wide ranges of salinity, temperature and dissolved oxygen levels. This fact has made its aquaculture possible under conditions that are difficult for other aquatic species (Oren, 1981). However, mass mortalities of these fish have also been reported worldwide in estuaries, rivers and coastal lagoons, often associated with low oxygen levels (Zamora-López et al., 2023; Baladia et al., 2025). In this sense, it is relevant for fish farmers a knowledge of the critical oxygen levels where the fish's regulatory mechanisms are unable to meet their metabolic needs, especially at high temperatures. This study describes the respiratory behaviour of the golden grey mullet (*Chelon auratus*) in response to gradual hypoxia at high temperature, paying special attention to the critical oxygen concentration and changes in ventilatory frequency.

## Material and methods

Goden grey mullets (*C. auratus*) from the Mar Menor lagoon (Murcia, Spain) were used. Two triplicate assays were carried out with two different weights and similar temperatures (A1: 3.5±0.1 g and 27.1±0.2ºC; A2: 11.02±0.18 g and 28.1±0.1ºC). Measurements were carried out in 33 and 160 L aquaria-respirometers, where 8 and 18 fish were kept for assays A1 and A2, respectively. When the fish were completely acclimated and showed a regular daily feeding rate (2 weeks), they were subjected to a 24-hour fast before measurements. Oxygen consumption ($MO_2$ in mgO2/kg/h) was determined simultaneously in the three replicates from the drop in oxygen concentration at 30-minute intervals after the water supply was stopped. For this purpose, an oxygen sensor (Hatch LDO101), a filter to remove excretion products, a small pump to homogenize the water, and a floating plastic film on the surface to prevent the exchange of oxygen with the air were placed in each aquarium. Ventilatory frequency (Vf) in beats/minute (bts/min) was video recorded and counted in 5 randomly selected fish at 30-minute intervals. The following data were obtained from each replicate: initial Vf at 100 % oxygen saturation (Vfini), maximum Vf reached by a fish (Vfmax), oxygen saturation (% Sat.) where Vf is significantly modified (Ovf), and critical oxygen concentration (Oc) where oxygen consumption decreases significantly ($P<0.05$). Oxygen levels were restored after reaching Oc.

## Results

Golden grey mullet behaved as a regulatory organism (Fig. 1), maintaining its oxygen consumption constant up to critical values of 30.6±6.0 % Sat. (1.97 $mgO_2$/l) and 37.2±2.2 % Sat. (2.36 $mgO_2$/l) for weights of 3.5 and 11.0 g, respectively ($P>0.05$). Initial Vf at levels close to 100 % Sat. ranged between 94±3 and 195±3 bts/min, with significantly higher values in smaller fish ($P<0.05$). The Vfmax reached was 168±8 bts/min and 234±11 bts/min for fish weighing 11.0 and 3.5 g, respectively ($P<0.05$). The fish responded to the decrease in oxygen concentration by significantly increasing the ventilation frequency from 67.2±7.6% Sat. (4.26 $mgO_2$/l) in 11.0 g fish at 28.1 ºC, although this value could not be obtained in the A1 assay.

## Discussion and conclusion

The results obtained suggest optimal oxygen values above 67 % Sat. at 28.1°C (4.26 $mgO_2$/l) where Vf and $MO_2$ remain constant. (Fig. 1). The extremely erratic behaviour and high variability observed in the Vf of the smallest fish made it difficult to obtain the Ovf in A1. These values are similar to those observed in other marine species from Mar Menor lagoon such as gilthead sea bream or sharpsnout sea bream, where values above 70% ensure the maintenance of metabolism without additional energy cost (Cerezo and García García, 2004).

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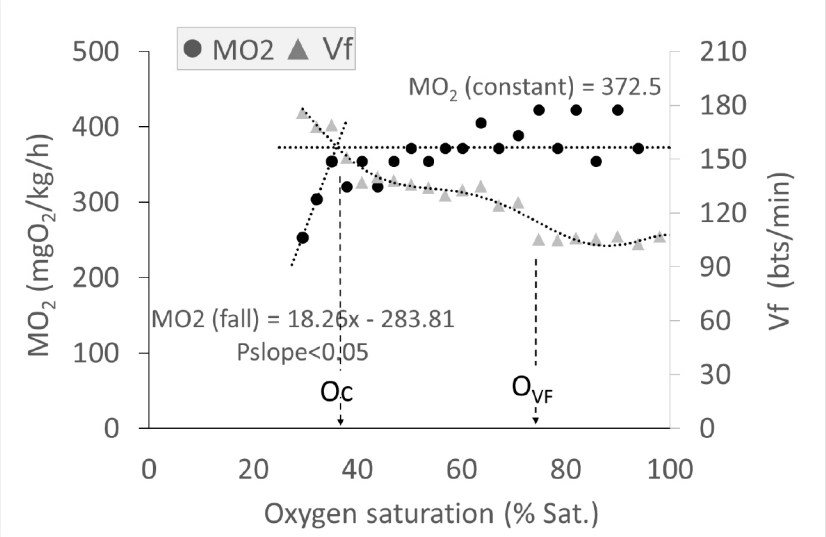


**Fig 1**. Oxygen consumption ($MO_2$) and ventilatory frequency (Vf) as a function of oxygen saturation in golden grey mullet (10.9 g; 28.3ºC). Critical oxygen saturation (Oc) and the point at which oxygen saturation modifies Vf (Ovf) are marked.

Oxygen values below 30.6 % Sat. (A1) or 37.2 % Sat. (A2) would be dangerous (altered Vf and $MO_2$), activating anaerobic metabolism and potentially causing the death of animal in the short term. These values were similar to the sharpsnout seabream (30.2% Sat. at 27.4°C; Cerezo and García García, 2004). Furthermore, in *Chelon auratus*, hypoxia not only generates an increase in Vf but also an increase in swimming activity as a response to find a better habitat. In contrast, these responses involve greater energy expenditure and a higher risk of exhaustion.

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# DEVELOPMENT AND EVALUATION OF A *Chlamydomonas reinhardtii*-BASED ORAL VACCINE AGAINST SALMONID ALPHAVIRUS (SAV2) AND VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) IN RAINBOW TROUT

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Aquaculture plays a critical role in meeting the growing global demand for food. However, the expansion of this industry is consistently challenged by infectious diseases, notably Salmonid Alphavirus (SAV) and Viral Hemorrhagic Septicemia Virus (VHSV), both of which significantly impact rainbow trout (Oncorhynchus mykiss) farming. Mortality associated with SAV infection varies widely, ranging from negligible levels to over 50% in severe outbreaks, whereas VHSV can cause mortalities of up to 90% (1,2).

In this study, we report the design, production, and preliminary evaluation of a novel oral vaccine targeting SAV2 and VHSV, developed using the green microalga *Chlamydomonas reinhardtii* as a biofactory. The antigenic region of the SAV2 E2 glycoprotein and fragment 16 of VHSV were selected and optimized for nuclear expression in *C. reinhardtii* through codon optimization and the strategic insertion of introns. The constructs were assembled using the Modular Cloning (MoClo) system and introduced into the algal nucleus via glass bead-mediated transformation. Western blot analysis enabled the identification of the most productive clone, which was subsequently scaled up and lyophilized to ensure stable antigen expression.

Vaccination was administered via oral intubation in rainbow trout, and post-vaccination responses were assessed by analyzing the expression of correlates of protection-associated genes and IgM titers.

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# RECOMBINANT GONADOTROPIN INDUCED OUT-OF-SEASON SPAWNING AND LARVAL CULTURE OF FLATHEAD GREY MULLET (*Mugil cephalus*)

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## Introduction

The flathead grey mullet (*Mugil cephalus*) has been recognised as an economically important euryhaline and eurythermal species with potential for aquaculture diversification in the Mediterranean and other regions, such as the Republic of Korea, Taiwan, and South Africa. The species positive attributes for aquaculture include good adaptation to captivity, rapid growth (Whitfield et al., 2012), omnivorous and detritivore feeding habits (Vinatea et al., 2018), and a valued food market including "bottarga" (the roe of fully mature females) with a market value exceeding 200 euros per kilogram. However, a bottleneck for mullet aquaculture development is reproductive control in captivity. Depending on the region and the broodstock holding system, reproductive dysfunctions have been reported to arrest reproductive development. In intensive systems mullet exhibited a severe reproductive dysfunction where the females fail to progress through the vitellogenesis phase and males fail to generate fluid sperm. Studies in IRTA have overcome these severe reproductive dysfunctions with a protocol of weekly injections of recombinant gonadotropins (rGth) (recombinant follicle stimulating hormone – rFsh and luteinizing hormone - rLh) to induce gametogenesis and spawning (Ramos-Júdez et al., 2022). The aim of this study was to use the rGth protocol to induce spawning outside the natural spawning period and further characterise the quality of gametes for larval culture of mullet.

## Material and Methods

The experiment was conducted between March 20th and July 31st, 2023, to induce gametogenesis in mullet using recombinant hormones. The experiment involved 15 mullet randomly selected from a stock of 38 wild adult mullet introduced into IRTA´s facilities (IRTA Aquaculture, Centre de La Rápita, Spain) between 2015 and 2018. Eight females and seven males were divided into an rGths (supplied by www.raraavis-bio.com) group that was treated species specific recombinant follicle stimulating hormone and recombinant luteinizing hormone and a control group treated with saline. The eight females selected for the study had 105.8 ± 5.81 $\mu$m oocyte diameter. The treatments of the females initiated on staggered dates, every two weeks a pair of one rGths female and one control female received the first recombinant hormone and saline respectively. The treatment of each pair commenced on March 20, April 3, April 17, and May 2, 2023. This programmed the spawning of the females to be every two weeks to facilitate live food production for the larval rearing. The rGths (rFsh and rLh) were administered to mullet following the protocol described by Ramos-Júdez et al. (2022). The eggs were incubated under constant conditions, and hatched larvae were transferred to different 100 and 500 L tanks. The larvae were reared with green water (microalgae *Tetraselmis Chuii* and *Isochrysis galbana*), rotifers, *Artemia* and Gemma wean diets (Skretting) based on protocols from Vallainc et al., (2021). Larval growth, development and survival were registered.

## Results & discussion

In the present study, both female and male mullet were induced with rGths to mature and spawn in June – July, a period outside the natural spawning season of wild populations, which typically occurs in September — October in the Western Mediterranean. All four females were during a 13-week period induced from the early stages of pre-vitellogenesis to complete maturation and ovulation. Over a 5-week period, all three rGth treated males developed from having no sperm present to producing and releasing sperm with spermiation sustained for 10 weeks. Three females spawned with males to give fertilised eggs that were incubated and larvae cultured. Juveniles were obtained with a maximum survival of 14.1 ± 5.2% to 42 days post hatch (final experiment day). In contrast, control females and males remained in the early stages of gametogenesis and did not produce viable gametes, highlighting the success of out-of-season induction with rGths. The present study has for the first time demonstrated that rGth hormones can be employed to induce gametogenesis, maturation and spawning of a teleost species

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out-of-season. Egg and larval quality were sufficient to show that, with further refinement, these protocols can be used by the aquaculture industry as a basis for juvenile production. The high efficacy of induced maturation, over 90% observed in the present and previous studies that developed the protocol, indicate that it offers an attractive alternative for breeder management. Therefore, the findings of this study hold crucial implications for aquaculture, as the culture of this species relies heavily on wild juvenile resources, with hatchery-produced juveniles being scarce.

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# THEORETICAL FRAMEWORK FOR A BIOECONOMIC MODEL, DESIGNED FOR A SEA BASS *(Dicentrarchus labrax)* AND FLAT OYSTER *(Ostrea edulis)* IMTA APPROACH IN THE ADRIATIC BASIN

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**Introduction**
Aquaculture can be considered a production activity in line with the Sustainable Development Goals (SDGs)/Agenda 2030, due to its important role for food security, nutrition, livelihoods, economies, and cultures. For this reason, it is important to develop pathways for improvement of the sector, that will allow further strengthening of aquaculture's role for contributing to the SDGs (Troell et al. 2023).

Marinet project is born with the scope of promoting and developing a sustainable, secure, and prosperous aquaculture in the Adriatic context.

To reach this scope several objectives were established in this project. One of them is empower small and medium-sized enterprises (SMEs) by sharing research, guidelines, and technological advancements and best practices.

To help fish farmers with decisional support, useful tools are being developed such as bioeconomic models. A bioeconomic-model is a mathematical tool capable of estimate the profitability of an organism production process (Garza, 2024), which in this project is intended to be possibly integrated to an Integrated multi-trophic aquaculture (IMTA). IMTA is the farming, in proximity, of aquaculture species from different trophic levels, and with complementary ecosystem functions (Chopin et al.2012).

The proposed bioeconomic model is developed following Chary et.al (2022) modeling process.

**Definition of the context**
As first step the context is defined. In this project IMTA involves Sea bass (*Dicentrarchus labrax*) and Flat Oysters (*Ostrea Edulis*), farmed in the Adriatic Sea. Offshore cage aquaculture approach is adopted for Sea Bass and Sea Bream farming, which represent the fed species in the IMTA ecosystem. Oysters represent the complementary part of the system, where their role could be defined as "organic extractive species". Among the positive interaction of these organism, we have an important mitigation of eutrophication, possible fed particulate waste reduction and potentially enhanced development of the oysters (Rusco et.al 2025) which can represent a valuable income diversification strategy (Jolly et Clonts, 1993).

**Formulation of the model**
The second step relates to process formulation and model selection, through the consideration of state variables and the equations available to possibly represent the production process. Variables and equation should be carefully chosen, keeping in consideration the context under study, the objectives, the available data and the desired complexity or generality of model among others (Chary et al. 2022).

To be in line with the needs of the farmers, the scope is to proposed an user friendly model, yet capable to provide pragmatic economic key performance indicators (KPI).

A bioeconomic model could be seen as a composition of interacting mathematical sub-models. In an aquaculture context it can be defined by three sub-models, biological, technological and economical (Garza 2024).

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Biological sub-model aims to predict parameters such as growth and survival, that in the proposed model will be calculated on the basis of Puerto et. al (2023) approach for the sea bass, and Kamiyama et al. (2021) approach for the oysters. These models were selected for their practical relevance to aquaculture and their integration of temperature as a variable, even though the original studies focused on different species, nile tilapia *(Oreochromis Niloticus)* in the former and cupped oyster *(Crassostrea Gigas)* in the latter.

In technological sub-model feed consumption is calculated through feed conversion rate (FCR) literature data (Zoli et al. 2023). Finally economical parameters such in particular costs and revenues are calculated to estimate economic indicators such as: break-even point quantity of production for both oysters and sea bass, profits and other economic performance indexes.

**Implementation and managerial implications**

The model will be implemented in a progressive web application (PWA), that will gather data recorded by farm sensors, farmer in field evaluation and market information. This data will be fed to the model for parameter estimation and they will be also used for model validation. Biological and technological model may be validated through linear regression, confronting predicted data and recorded data (Brigolin et al. 2010). Currently Marinet team, through his AI experts, is also working on the integration of AI in the PWA which will be used to optimize the Bio-economic model.

**Conclusions**

The key feature of the proposed framework is its ability to demonstrate how both the sea bass and flat oyster farming influence the overall economic viability of an IMTA (Integrated Multi-Trophic Aquaculture) farm. Data can be incorporated either from scientific literature or directly from farm records.

It is important to note that this bio-economic model, as a preliminary approach, remains largely theoretical. To enhance its practical applicability, further development is required, including the organization of focus groups with farmers to identify potential modifications and integrations.

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# AN OVERVIEW OF THE CURRENT IRISH MARINE AQUACULTURE ENVIRONMENTAL INSITU MONITORING AND FORECASTING SYSTEMS READILY AVAILABLE TO THE INDUSTRIES AND THE NEED FOR FUTURE DEVELOPMENT

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Introduction

The Irish Marine Aquaculture industry, spread out in over 282 sites throughout the entire coastline, representing both Shellfish and Finfish ventures, is significantly important to the Irish economy. The potential occurrence of 'Jellyfish blooms', Harmful algal blooms (Habs) and Harmful algal events (Haes) present a continuous challenge to these industries. Annual and seasonal occurrence of growth and transportation of problematic species to aquaculture need to be monitored and potentially predicted in a more integrated manner yet with specific requirements of outputs for each industry. The most recent 2 years proved particularly less biologically and financially problematic in terms of presence of levels of toxic species of algae for the shellfish industries. The Irish salmon industry experienced particularly difficult biological challenges in 2023 and 2024 and '..addressing these challenges remains a priority to stabilise and improve salmon production'(BIM,2023). Through engagement with stakeholders and state bodies, the following overview details the most used insitu environmental monitoring tools and early warning systems and the need for continued improvements.

# ASSESSING WELFARE AND QUALITY IN ATLANTIC HALIBUT (*Hippoglossus hippoglossus*) SLAUGHTERED USING THE *IKI JIME* METHOD

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## Introduction

*Iki jime* (also spelled *ike jime*), or brain spiking, is a traditional Japanese method of fish slaughter that involves humane killing through a quick spike to the brain, followed by inserting a thin wire along the spinal column to halt muscle reflexes. This technique aims to extend the pre-rigor period and preserve high fish quality and freshness. However, limited research exists on its welfare and quality implications, and its application on an industrial scale can be challenging. This study evaluated welfare and quality parameters in Atlantic halibut (*Hippoglossus hippoglossus*) slaughtered using the *iki jime* method, compared with the conventional stunning method used in commercial practice.

## Materials and Methods

Due to Norwegian slaughter regulations, the traditional *iki jime* method involving direct brain spiking is not permitted. Therefore, this study adapted the procedure by performing *iki jime* after approved stunning and bleeding methods. While applying the *iki jime* method after stunning, the thick cranial structure of the halibut also caused challenges in wire insertion through the brain. Instead, the tail was removed, and the wire was inserted from the posterior end along the spinal column toward the brain.

In the first study, 20 Atlantic halibut (weight excluding tail: 4.5±0.7 kg) were individually captured using dip nets and percussively stunned. The control group (n=10) were gill-cut and bled in ice slurry. The experimental group (n=10) underwent the *iki jime* method, involving tail removal, wire insertion from the back, and bleeding in ice slurry. Blood samples were collected immediately after the stunning. Postmortem pH and rigor index were monitored for 72 hours. All fish were stored in ice-filled boxes throughout the period.

In the second study, 18 Atlantic halibut (weight excluding tail: 3.5±1.2 kg) were obtained via commercial procedures involving crowding, pumping, stunning and bleeding onboard a vessel. The control group (n=6) was dip-netted from the sea pen and percussively stunned. The remaining fish were pumped onboard the vessel, where one group was subjected to electrical stunning (n=6), and the other to percussive stunning (n=6). All fish were subsequently subjected to the *iki jime* procedure as described above. Blood samples were also collected, and postmortem pH and rigor index were monitored for 72 hours.

## Results

The first study observed no significant differences between the groups in postmortem pH or plasma cortisol levels. Plasma cortisol concentrations were 25.0±19.1 (control) and 35.1±10.1 ng/ml (*iki jime*). Both groups exhibited an initial muscle pH of 7.3±0.1, which declined to 6.1-6.2 after 72 hours. Maximum rigor at 24 hours postmortem.

Similarly, the second study showed no statistically significant differences in postmortem pH, rigor index, or plasma cortisol levels among the groups. Cortisol concentrations averaged below 40 ng/ml across all treatments. Initial pH values were 7.1±0.1 for the control group and 6.8±0.4 and 6.8±0.1 for the percussive and electrically stunned groups, respectively. By 72 hours postmortem, pH values in all groups had declined to between 6.1 and 6.2. Maximum rigor was also reached within 24 hours in all groups.

## Conclusion

The studies demonstrated that the *iki jime* method, while traditionally associated with superior product quality, does not confer significant advantages in welfare or slaughter quality relative to the conventional slaughter methods used in halibut processing. Plasma cortisol levels remained low across all groups, indicating that the slaughter procedures used were gentle and did not induce high stress levels. Therefore, the commercial slaughter methods today deliver both welfare outcomes and product quality comparable to those achieved with the *iki jime* technique.

# SUB-CHILLING AQUACULTURE SPECIES FOR IMPROVED QUALITY AND SHELF LIFE

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## Introduction

Sub-chilling is a preservation method that maintains the freshness and prolongs the shelf life of seafood by reducing the temperature to between 0 °C and its initial freezing point, for instance, from -1 to -1.5 °C. This process keeps the fish in a fresh state, rather than frozen. Sub-chilling can be achieved in several ways. Refrigerated seawater (RSW) is frequently used for whole fish, while research on sub-chilled fillets often employs impingement or cryogenic freezing. In the aquaculture industry, sub-chilling fish in RSW onboard vessels immediately after slaughter by the pens allows the delivery of fish already sub-chilled at the early stages of the value chain.

Research also indicated that sub-chilling eliminates the need for ice and reduces meltwater incidence (Rotabakk et al., 2023). Consequently, more fish can be packed and maintained at sub-chilled temperatures until reaching the market, significantly reducing greenhouse gas emissions and costs (Iversen et al., 2021). This presentation provides an overview of studies on sub-chilling for various aquaculture species in Norway.

## Materials and Methods

The studies collectively evaluated the effects of sub-chilling of whole fish in RSW on the quality and shelf life after processing and storage. The species studied included Atlantic salmon (*Salmo salar*), Atlantic halibut (*Hippoglossus hippoglossus*), brown trout *(Salmo trutta*), and Arctic char (*Salvelinus alpinus*).

Whole fish were subjected to sub-chilling in RSW, with the control group being fish stored on ice, the standard method used today. The duration of RSW storage varied by species: salmon for 4 days, and halibut, trout, and char for 1 day. These fish were then stored further with or without ice before processing. Salmon were filleted on day 7, portioned, and packaged in modified atmosphere (MA) (60% $CO_2$:40 % $N_2$), then stored at sub-chilled conditions at -1 °C. For the study on trout, temperature abuse was introduced to mimic possible transport challenges. Quality parameters measured included weight changes, water holding capacity, salt uptake, colour, texture, sensory (odour test, Quality Index Method), and microbiology shelf life.

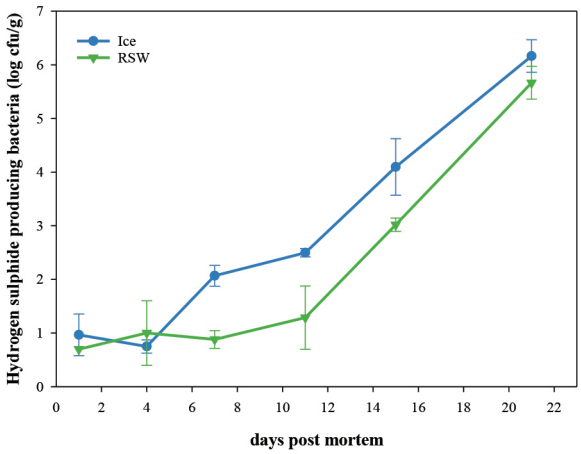


Fig.1: Graph of hydrogen sulphide bacterial growth for Arctic char sub-chilled in RSW for 1 day versus ice-storage.

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**Results**

Our studies showed that storing gutted salmon sub-chilled in RSW for 4 days resulted in a 0.7-0.9% net weight gain, with improved water holding capacity and higher salt uptake compared to ice storage (Chan et al., 2021; Chan et al., 2020). Additionally, the sensory evaluation (off-odour) and microbiological shelf life were significantly increased, at least doubling during sub-chilled storage packaged in MA (Chan et al., 2024).

For trout, storing in RSW for 1 day also resulted in weight gain, although this effect subsided afterwards. Despite experiencing temperature fluctuations during transport, the quality and shelf life of sub-chilled trout were comparable to those stored on ice. Sub-chilling halibut in RSW for 1 day, followed by packing in boxes with and without ice, extended the microbiological shelf life by 5 days compared to 17 days for the control group. This also corresponds with improvements in sensory analysis (QIM). Moreover, sub-chilling halibut showed a positive effect in mucus removal. Storing Arctic char sub-chilled in RSW for 1 day and keeping them in boxes without ice gave similar microbial growth as the control group. Nevertheless, the growth of hydrogen sulphide-producing bacteria was lower with RSW sub-chilling (Fig. 1).

**Conclusion**

Our studies have demonstrated that sub-chilling in RSW is an effective method for enhancing the quality and extending the shelf life of various fish species. This can be combined with other technologies and contribute to reducing food loss. Therefore, it is possible to package fish under sub-chilled conditions without ice, provided the temperature is properly controlled.

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# QUANTITATIVE PROTEOMIC ANALYSIS OF ATLANTIC SALMON *Salmo salar* BRAIN REGIONS, HEAD KIDNEY, AND SPLEEN IN RESPONSE TO ELEVATED WATER TEMPERATURE

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**Introduction**

Rising summer sea temperatures, driven by climate change, pose a significant challenge to the salmon aquaculture industry. Prolonged exposure to temperatures above the optimum range adversely impacts appetite, growth, metabolism, and disease resistance. Although the physiological effects of thermal stress on fishes are well-documented, there is limited knowledge on the molecular mechanisms underlying the thermal stress response in the fish brain. The brain, as the central organ regulating the stress response through the hypothalamus-pituitary-interrenal (HPI) axis and the brain-sympathetic-chromaffin cell (BSC) axis, is highly sensitive to environmental stressors. Moreover, it is a complex organ comprising distinct regions with specific functions, including growth, reproduction, cognition, movement, and metabolism. Investigating various brain regions of salmon exposed to thermal stress conditions would provide insights into region-specific and common stress adaptation mechanisms. Therefore, in this study, we investigated the thermal stress impacts on cerebellum (CBE), hypothalamus (HYP), medulla oblongata (MED), optic tectum (OPT), pituitary gland (PIT), and telencephalon (TEL) of salmon using quantitative proteomics. Additionally, we examined the proteomic profiles of two immune organs, the head kidney (HK) and spleen (SP), to elucidate their roles under thermal stress conditions.

**Materials and methods**

A total of 150 Atlantic salmon post-smolts (mean weight: ~300 g) were randomly allocated to six 500 L seawater flow-through tanks. Three tanks were assigned to the control group (maintained at 15°C) and three to the thermal stress (TS) treatment (temperature increased by 1°C over four weeks and then maintained at 19°C for four weeks). The dissolved oxygen saturation was maintained at 100 % ± 5 % throughout the experiment in both control and TS treatment tanks. At the end of the experiment, six distinct brain regions -CBE, HYP, MED, OPT, PIT, and TEL were collected from six fish per treatment. Additionally, HK and spleen SP were collected from five fish per treatment. Proteomic analysis of samples was conducted using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). Following LC-MS/MS analysis, statistical analysis (p-value $<0.05$, fold change $> \pm 1.5$) were performed to identify differentially expressed proteins (DEPs) between the control (15°C) and TS group (19°C). Finally, bioinformatics tools (DAVID & ClueGO) were used for functional enrichment analysis of the DEPs to identify gene ontology terms and KEGG pathways.

**Results**

An average of over 9000 proteins were identified across the six brain regions. A comparative analysis of DEPs indicated that only two proteins were common across all regions, with the majority of DEPs being unique to specific regions. SERPIN H1 (heat shock protein 47), a well-validated thermal stress biomarker was found to be upregulated in the TEL, HYP, and PIT regions of the TS group. Proteins related to several key pathways and processes, such as amino acid metabolism and protein folding in PIT; endopeptidase activity in TEL, HYP, and PIT; and cardiac muscle contraction, RNA binding, and glucose metabolism in MED, were upregulated. Furthermore, in most regions except MED and OPT, proteins involved in mRNA processing, cytoplasmic translation, spliceosome, RNA binding, and ribosome were downregulated.

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In HK and SPL, the molecular chaperones SERPIN H1 and HSP90a were upregulated under TS conditions. Bioinformatics analysis revealed that upregulated proteins in HK were enriched in unfolded protein binding and protein processing in the endoplasmic reticulum. In the spleen, notable changes were observed in proteins related to lipid metabolism and protein degradation. Consistent with the brain regions, proteins downregulated in both the spleen and HK were linked to pathways and processes associated with translation and transcription.

**Conclusion**

This study represents the first investigation of the thermal stress response of distinct brain regions (CBE, HYP, MED, OPT, PIT, and TEL) in Atlantic salmon at the proteome level. The differential expression analysis revealed that protein expression varies among regions in response to sub-optimal water temperatures, suggesting that each region may possess a unique adaptation mechanism to cope with thermal stress. Notably, the upregulation of SERPIN H1 across multiple brain regions, as well as in HK and SPL, underscores its critical role in protecting cells from thermal stress-induced damage. Additionally, the downregulation of the transcription and translation machinery in all tissues indicates a possible reduction in the efficiency of the splicing process.

Overall, this proteomic study provides an enhanced understanding of the tissue-specific molecular mechanisms underlying thermal stress adaptation in salmon. These insights have the potential to support the industry in enhancing the resilience of salmon populations to increasing water temperatures induced by climate change. Furthermore, our region-specific brain proteome analysis may serve as a foundational basis for future research investigating the role of distinct brain regions on various physiological processes including behaviour, cognitive function, appetite regulation, energy metabolism, and neuroendocrine responses under varying environmental conditions.

# EVALUATING NUTRIENT CIRCULARITY IN INTEGRATED AQUACULTURE SYSTEMS: CRITERIA AND INDICATORS

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Nutrient circularity is an application of circular economy principles that addresses issues such as nutrient accumulation or loss, and a shift away from fossil and synthetic fertilisers. The concept is increasingly being explored and is relevant for managing nutrients more sustainably in agri- and aquaculture systems, but the standards by which nutrient circularity can be evaluated require further clarification, and quantitative indicators should be established. Identifying the nutrient circularity performance of integrated aquaculture systems is particularly relevant to better understand how the combination of multiple and complementary species farmed or naturally present in these systems can upcycle nutrients from waste.

The main objectives of this study were to improve understanding of nutrient circularity and clarify how to quantify it for (integrated) aquaculture systems. To this end, criteria for describing nutrient circularity were first defined based on a literature review, and quantitative indicators were then identified for each criterion to create an indicator framework. Finally, this framework was applied to three contrasting experimental integrated aquaculture systems (i.e. aquaponic, biofloc and polyculture pond) and their conventional monoculture system counterparts from previous studies to test its ability to compare nutrient circularity in aquaculture systems.

Six complementary criteria (and 21 associated indicators) for describing nutrient circularity were identified: productivity, efficiency, self-sufficiency, recycling, regeneration, diversity and complementarity. These criteria, related to circularity principles, provided a clear framework for evaluation. Application of the framework indicated that the integrated systems evaluated usually outperformed conventional monoculture systems, which highlighted the potential of integrated systems to manage nutrients more sustainably. These contrasting integrated systems showed that different pathways (e.g., microbial loops, complementarity between farmed species) can be mobilised to create and (re-)cycle nutrients. Although relatively simple indicators were developed, lack of data prevented quantification of several indicators and thus a full comparison of the systems. Overall, this study helps clarify the concept of nutrient circularity and supports the development of integrated farming systems for more sustainable use of nutrients.

**Full reference**

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# DEVELOPMENT OF STANDARDIZED *Enterocytozoon hepatopenaei* (EHP) CHALLENGE MODELS FOR VALIDATING DISEASE CONTROL TECHNOLOGIES IN PENAEID SHRIMP (*Penaeus vannamei*)

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*Enterocytozoon hepatopenaei* (EHP) has become a persistent and economically devastating pathogen in shrimp aquaculture, especially in Asia. The inconsistency between field outcomes and laboratory trials has posed major challenges for the effective evaluation of disease control strategies.

In response, ShrimpVet Laboratory has developed and refined a series of standardized EHP challenge models designed to address this critical gap. This presentation outlines the design, optimization, and validation of three laboratory-scale EHP challenge protocols, grounded in real-world epidemiological observations and disease expression patterns from the Mekong Delta region. These include: **(1)** a cohabitation challenge model using spore-shedding donor shrimp to mimic chronic infections; **(2)** a per os challenge model using homogenized feces collected from EHP-injected shrimp, with a known density of spores, to ensure standardized oral exposure; and **(3)** a combination model, integrating EHP challenge with the induction of White Feces Syndrome (WFS) to better mimic complex, multifactorial field scenarios commonly observed in commercial shrimp production.

Quantification of infection intensity is performed through molecular diagnostics (qPCR), with evaluation of model reproducibility, host response, and sensitivity to treatment efficacy. These models have since been applied to screen a wide range of novel functional feed additives, probiotics, and microbial solutions under standardized yet field-relevant conditions.

By reproducing realistic infection dynamics, ShrimpVet's platform enables precise benchmarking of candidate technologies prior to field deployment. The standardization of EHP challenge models represents a critical advancement for the shrimp health sector, offering a robust and scalable platform for validating next-generation disease management tools. This work supports both innovation and resilience in sustainable shrimp farming systems.

# MOLECULAR PATHWAYS INVOLVES IN MATERNAL IMPRINTING OF EUROPEAN SEA BASS IMMUNITY

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## Introduction

The rapid growth of aquaculture and the intensive culture conditions are leading to chronic stress situations favoring infections including those caused by viruses (Kibenge et al., 2019). Betanodavirus (NNV) is one of the main viral threatens in the Mediterranean coast. In larvae and juvenile stages, NNV infection reaches mortality rates up to 100%, but adults are asymptomatic carriers of infection. NNV can be both horizontal and vertically transmitted (Bandin & Souto, 2020). Focusing on vertical transmission, we must consider the ability of NNV to reach the gonad and establish a latent infection and the transmission of NNV through gametes to their progeny, which is highly susceptible (Valero et al., 2018a). Up to date, vaccines are the preventive method more explored. Although, maternal transfer of immune factors, defined as the transfer of innate or adaptive immune factors by an immunocompetent female to an immunologically naïve neonate has been described in fish (Swain & Nayak, 2009). This process could not explain why female vaccination improve the development of the immune system of the progeny in terms of gen expression up-regulation without promoting the transfer of maternal mRNAs, as recently demonstrated (Valero et al., 2023). Thus, maternal immune priming has been proposed to influence the immune development of the progeny in fish (Valero et la., 2023). However, nothing is known about the molecular pathways that orchestrated this issue. The objective of this study is to identified expression pathways modulated by females' vaccination in both ovary and fertilized eggs using massive transcriptomics.

## Material and methods

The females' specimens of a mature broodstock, but not the males, were vaccinated against NNV with and effective vaccine (pBAD), produced and characterized previously (Gonzalez-Silvera et al., 2019)mortalities in aquaculture industry can reach up to 100%, some species being especially sensitive as is the case of European sea bass (Dicentrarchus labrax and a similar group but sham-vaccinated was established as control. Serum and ovary samples from vaccinated or sham-vaccinated females were obtained 21 days from the vaccination. Several pools of 0.08g of early fertilized eggs, 0 days post-hatching (dph), were obtained from vaccinated and sham-vaccinated broodstocks one year after females' vaccination. Also, larvae from 4 and 9 dph were sampled. Anti-NNV IgM levels were analyzed in females' serum samples (Valero et al., 2018b). Ovary and egg fertilized samples were submitted to mRNA-seq (García-Beltrán et al., 2024). Also, ovary, egg and larvae samples were used to analyze beta-defensins by qPCR (García-Beltrán et al., 2024).

## Results

The efficiency of vaccination was confirmed by the production of anti-NNV IgM antibodies in vaccinated females, but not in the sham-vaccinated group. Transcriptomic analysis revealed the following differentially expressed genes (DEGs): 245 up-regulated and 3179 down-regulated genes in ovary while 3143 up-regulated genes and 4773 down-regulated genes in fertilized eggs, comparing in all cases between control and vaccinated groups. In ovary, there were six KEGG pathways up-regulated: ribosome, oxidative phosphorylation, spliceosome, cell cycle, oocyte meiosis and proteasome. In egg fertilized, there were ten KEGG pathways up-regulated including spliceosome, RNA degradation and mRNA surveillance pathway. Bdef expression was up-regulated at 4 and 9 dph in eggs fertilized from vaccinated females compared to controls.

*(Continued on next page)*

**Discussion and conclusions**

None of the canonical immune pathways resulted altered. This issue together with the transcriptional study of Bdef revealed that their expression increased in larvae from vaccinated-females without a detectable maternal transfer of mRNAs as previously described (Valero et al., 2023). However, we detected some up-regulated pathways that could be related to immunity and viral infection resolution such as oxidative phosphorylation (Li et al., 2019) or spliceosome (Chang & Zhang, 2017). Moreover, other up-regulate pathways such as RNA degradation and mRNA surveillance might be orchestrating the previously observed effect of higher levels of expression of immune-related genes in the progeny from vaccinated-females. In fact, RNA degradation and mRNA surveillance pathway are considered to exert antiviral roles (Balistreri et al., 2017). In conclusion and although further studies are mandatory to ascertain all the molecular mechanism involved, our data suggest that general molecular regulation pathways of the cells might be the clue for understanding the maternal priming of immunity in the fish progeny.

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# DIAGNOSIS OF NNV LATENT INFECTION IN THE GONAD OF GREATER AMBERJACK BROODSTOCKS SHOWS A SEX-BIASED IMMUNE RESPONSE AGAINST INFECTION

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**Introduction**
In recent years, population food demand has been greatly increasing making necessary to diversify marine species to widen the offer to consumers. In this context, greater amberjack (*Seriola dumerili*) constitutes a serious candidate to be introduced in the Atlantic and Mediterranean aquaculture (APROMAR, 2023). However, knowing greater amberjack susceptibility to pathogens is crucial to assess the culture economic viability. One of the most harmful threats to worldwide aquaculture is betanodavirus (NNV). NNV causes high mortality rates in larval stages but causes asymptomatic infections in adult specimens in which NNV triggers a latent infection in their gonads (Bandin & Souto, 2020). Thus, NNV can be transmitted to their highly susceptible progeny (Valero et al., 2018). Therefore, we aimed to find non-lethal NNV-diagnosis methods involving gonadal fluids to detect latent or persistent infections and deeper in the differential immune response triggered in ovary and testis.

**Material and methods**
Adult' specimens of greater amberjack were naturally infected with NNV but remained asymptomatic. Ovarian biopsy, sperm and blood samples were obtained. The expression levels of RNA-dependent RNA-polymerase, *rdrp*, and protein capsid, *cp* were analyzed by qPCR in ovarian and sperm samples following previous protocols (Valero et al., 2015). Also, luciferase activity using RTG *psaumx2-luc* cells was measured in blood samples. Several genes related to antiviral response, antimicrobial peptides (AMPs), inflammation and cell markers were studied by qPCR. Statistical differences between control and infected males or females were studied by Student-t test when normality was assumed and U-Mann Whitney test when data did not meet parametric assumptions.

**Results**
Gene expression analysis of viral markers in gonadal fluids and luciferase activity in blood analysis revealed that 11/14 males were positive for NNV infection while only 5/13 females were so. Nevertheless, qPCR could not detect viral markers in blood while in gonads luciferase activity was undetectable. Regarding the immune response, we observed a great up-regulation of *mx*, marker of the interferon route in males, but no in females. None of the immune related genes were altered in females. In males, T cytotoxic and T helper cells encoding genes were extremely up-regulated. Similarly, AMPs such as histones, hepcidin, lysozyme or complement factor C3 encoding genes were highly up-regulated in testes.

**Discussion and conclusions**
Our data revealed that qPCR is a sensitive method to detect NNV markers in gonadal fluids but not in blood while luciferase method works in blood samples but not in gonadal fluids. These findings lead us to establish new effective non-lethal diagnosis protocols for NNV. Strikingly, great differences in immune response were observed between males' and females' gonads. The lack of immune response triggered in ovary might be a consequence of their physiology as previously suggested (Dias & Brouwer, 2022). Moreover, the generalized tendency to up-regulation of most of the immune markers in the testis point to a persistent infection instead of a latent infection.

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# GONADAL MORFOLOGY THROUGH THE FIRST TWO YEARS OF AGE OF *Ostrea edulis* CULTURED IN A SALINE ENVIRONMENT

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## Introduction

The European flat oyster (*Ostrea edulis*) formed a large population of more than 100 million individuals in the Mar Menor (MM) during the 1980s and 1990s. However, its population declined to 6 million individuals in 2006 (Rosique, 2006). Currently, there is an initiative to recover *O. edulis* populations in the MM lagoon as one of the candidate species to mitigate microalgal proliferation caused by anthropogenic eutrophication of the lagoon and promote improvements in water quality and restore its ecological functionality. One of the most important biological processes for maintaining populations is reproductive function and within its puberty, understood as the production of the first wave of gametes is the most critical processes. Although extensive research has been done in adult specimens, gonadal development in the flat oyster during the first reproductive period after attachment to the substrate is poorly understood, with only one histological study available (Cole, 1942). The objective of this study is to understand the gonadal development of the *O. edulis* oyster cultivated and maintained in the MM lagoon, at the histological level, in order to promote oyster cultivation for both future restoration and bioremediation purposes in a lagoon with eutrophic conditions.

## Material and methods

The oysters used in the experiment were obtained from the reproduction of wild specimens collected from the MM lagoon in 2022 (Hernandis et al., 2023) and kept at the Oceanographic Center of Murcia (COMU-IEO), CSIC. Once the spat was obtained at the hatchery and after reaching a size > 20 mm, the sexually immature specimens were transferred to the entrance channel of the San Pedro del Pinatar salt flats, located in the northern basin of the lagoon. This channel, 14 m wide and 330 m long, is supplied daily with water from the lagoon itself, so its conditions are similar to those of the lagoon. The oysters were placed in oyster baskets stacked high off the bottom on cement blocks. Samples were taken to analyze gonadal development (n = 30) using conventional histology (García-García et al., 2016) during two years once a month.

## Results and Discussion

Our results showed that specimens under a year of life had follicles with spermatogenic activity during two seasons of the first year, around May and around November. However, the biggest development of the gonad in terms of gametogenic tissue proportion was observed during May. During the second year of age, we observed the same pattern of gonadal development, but the percentage of gonadal tissue and the time that the gametogenesis stage lasted were higher. However, those parameters during the autumn developmental season kept steady during both years. Moreover, during the gametogenetic autumn stage, no females were observed at any of the two years analyzed. These data are contradictory with previous observations that determined a recruitment spat season during autumn in Mar Menor lagoon (Cano et al., 1997). However, as the population study correspond only with the first two year of live, further studies with mature specimens are needed to clarify this issue.

Regarding gonad morphology, in general, we observed that the majority of individuals presented a gonad composed by follicles where a certain spermatogenic gradation was observed from the inner most part where the most undifferentiated cells were located towards the gonoduct. Among the epithelial cells of the gonoduct, the presence of acidophilic cells stood out. During the first years, only a single specimen in May presented previtellogenic oocytes in the periphery of the follicular acini described as phase 3 of development by Cole (1942). In the second year of life a spring gametogenetic stage occurred with higher percentages of females (second vs first year: 26% vs 10%). Indicating this that females' development need a progressive ontogeny, probably related to hormonal axis development. Issue that has not been properly studied until now and is being analyzed in our laboratory. Although little is known about pubertal development in the oyster, a previous study (Cole, 1942) described a development similar to that found in the oyster from the MM lagoon.

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**Conclusions**

The gonadal morphology observed during the first two years of life of *Ostrea edulis* pointed to the spring season as the main reproductive one, although a slight development was also observed in autumn. As expected the capability to produce gametes was progressively increased through the first two years of life, as revealed by the increase of the gonadal area through time. Interestingly, the proportion of females detected was also higher in the second year compared to the first one.

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# ASSESSING TROUT FARMING IMPACTS ON RECEIVING WATERCOURSES: SELECTION OF THE MOST EFFECTIVE MACROINVERTEBRATE AND DIATOM METRICS

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**Introduction**

Mountain aquatic ecosystems, due to their oligotrophic characteristics, are particularly sensitive to human activities. Inland trout farming can affect receiving watercourses by increasing nutrients (especially nitrogen and phosphorous compounds), organic matter and suspended solids, as well as through the release of drugs and chemicals. The extent of these impacts depends on several factors, such as farm size, the volume of water diverted, and the self-purification capacity of downstream waterbody. Furthermore, these effects can vary spatially and temporally, influenced by farming practices, stocking densities, feed quality and hydrological conditions. Local impacts are typically observed up to a kilometer downstream of the trout farm, but occasionally, they can extend to a regional scale.

In the Province of Trento, trout farming constitutes a significant production sector with approximately 60 trout farms mainly focused on the rearing of rainbow trout (*Oncorhynchus mykiss*). In this context, the project "Filiera ASTRO: Competitiveness and Sustainability of Mountain Aquaculture" (CUP J38H23001450007), funded by "Piano Nazionale Complementare – PNC" of the Ministry of Agriculture, Food Sovereignty and Forests (MASAF), aims to improve the local aquaculture industry through the reuse of fish farming by-products, and applying new technologies to reduce feed waste and environmental impacts. The project framework consists of four work packages (WP).

The main objectives of WP2 (Environmental sustainability of aquaculture and water courses quality) are: (I) assess specific aquaculture impacts on receiving watercourses, (II) evaluate innovative methods for biological communities (traditional and metabarcoding approaches), (III) identify the best analysis tool to assess the impact of aquaculture on receiving watercourses and (IV) assess efficacy of mitigation measures.

**Methods**

In summer 2024, water quality of the receiving water courses was monitored for a group of 16 trout farms using freshwater macroinvertebrates (Indice Biotico Esteso, IBE) and diatom index (ICMI, Italian Water Framework Directive 2000/60/EC method). Diatom communities were further categorized into ecological guilds to provide insights into trophic conditions. To estimate nutrient enrichment, periphyton samples were collected for chlorophyll-a concentration and ash free dry mass analysis. Water samples were also collected at upstream and downstream stations to retrieve physicochemical parameters (e.g. $O_2$ saturation, COD, $BOD_5$, total phosphorus, nitrate-N, ammonium-N, *Escherichia coli* concentration) and to calculate LIMeco (Italian WFD 2000/60/EC method) and LIM indices.

Farms monitored in this study are characterized by a flow-through scheme, which is widely applied in Trentino, due to rapid water renewal, optimal oxygen concentration and suspended solids removal, which allow optimal rearing conditions for fish. In some facilities, liquid oxygen is applied to support the production, and effluent treatment systems have been implemented to minimize environmental impacts.

Three trout farms that will implement drum filters within the project were selected to assess the mitigation potential. In addition to the previously described methods, quantitative analyses of the macroinvertebrate community were also performed. Five Surber net replicates were collected for one upstream and one downstream sampling station, and macroinvertebrates were identified to genus/family level. STAR-ICMi index, the official Italian method based on freshwater macroinvertebrates under WFD, was also calculated.

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Main compositional (e.g. 1-GOLD, ASPT, EPT, EPTD, taxa richness, Shannon index) and functional feeding group metrics (e.g. percentage of grazers, shredders, collectors, Shredders/Collectors ratio) were calculated to identify those most sensitive to trout farm impacts. Differences between upstream and downstream stations values were assessed using the Mann-Whitney U test.

**Results and Discussion**

IBE results from the summer 2024 campaign indicate that most upstream stations were in 'Quality Class I', with limited changes observed at downstream stations (where the lowest class recorded is II). These findings agreed with the preliminary ICMI results, showing little or no impact on receiving watercourses. Diatom guild analysis further supported these conclusions, revealing the dominance of oligotrophic and low-profile taxa, which are typical of low-nutrient, and fast-flowing environments.

In all three farms that will apply impact-mitigation measures, %OC (percentage of oligochaetes and chironomids) showed a significant increase observed at downstream stations. In the two most impacted sites (based on IBE), this is accompanied by a decrease in 1-GOLD, ASPT, and percentage of grazers, along with an increase in the percentage of filter feeders. These trends suggest a community shift toward pollution-tolerant taxa feeding on fine particulate organic matter derived from aquaculture effluents, corroborating existing literature.

LIMeco assessments placed most stations in 'Quality Class I', with only three showing lower quality (the lowest being Class IV). LIM appeared to be more stringent, with nearly half of the stations classified as Class II and the rest as Class I. Among chemical parameters, a general increase in total phosphorus and total ammonium concentrations were observed at downstream stations, likely attributable to fish excreta and uneaten feed.

**What's next?**

For the three selected trout farms, biological and chemical analyses will be repeated under different seasonally conditions before-and-after the installation of drum filters. These data, along with a bulk eDNA metabarcoding approach, will be used to assess the effectiveness of the mitigation measures, understand how these methodologies can be integrated and develop new metrics and indices that are specifically suited to detecting trout farming-related impacts in montane lotic ecosystems.

# FROM LAB TO FARM: VALIDATING AN ORGANIC ACID BLEND FOR BACTERIAL DISEASE MANAGEMENT IN FISH

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Gram-positive bacterial diseases, particularly those caused by *Lactococcus* and *Streptococcus* species, cause significant economic losses in fish farming. This study evaluated the efficacy of a functional organic acid blend (Bacti-Nil Aqua, Adisseo) against these pathogens in multiple levels through *in vitro*, laboratory, and field trials.

*In vitro* assays determined the minimum inhibitory concentration (MIC) against field isolates of *Lactococcus garvieae*, *Lactococcus petauri*, *Carnobacterium sp*., and *Streptococcus penaecida* from Brazil, Colombia, and the Mediterranean region. The MIC values ranged from 1,000 to 4,000 ppm, confirming consistent antimicrobial activity at commercially feasible concentrations. Subsequently, *in vivo* challenge trials demonstrated the additive's protective effects. In Nile tilapia (*Oreochromis niloticus*) challenged with *Aeromonas* and *Streptococcus*, dietary supplementation reduced final mortality by 22%. Similarly, in rainbow trout (*Oncorhynchus mykiss*) challenged with *L. garvieae*, supplementation lowered mortality by 40% compared to controls ($P<0.05$). These findings were validated in a commercial tilapia farm trial in Thailand, where supplementing the diet with 0.2% of the blend improved the FCR by 3%, increased total biomass by 5%, and yielded a 3.5 return on investment (ROI).

These integrated results confirm that the additive provides consistent antimicrobial benefits across diverse species, environments, and production stages. Its demonstrated efficacy in both laboratory and field settings establishes its value as a practical tool for managing bacterial diseases in aquaculture.

# DIETARY PHYTOBIOTIC ACCELERATES SKIN WOUND HEALING IN A ZEBRAFISH LASER ABLATION MODEL

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## Introduction

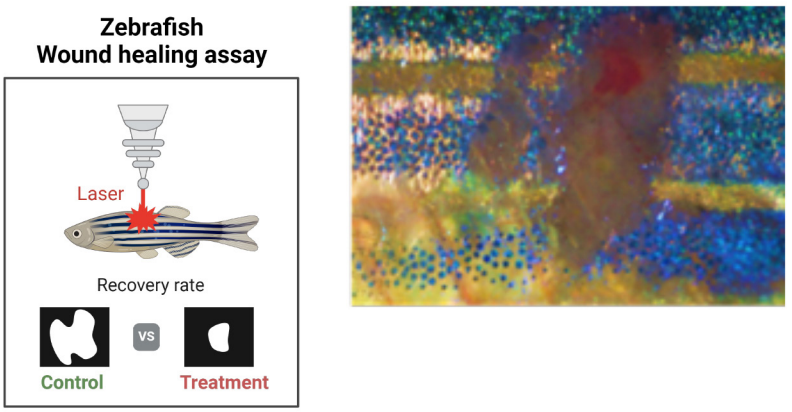
Fish skin functions as the primary barrier against pathogens, mechanical damage, and environmental stressors, playing a crucial role in physiological homeostasis via mucus secretion and osmoregulation. Under intensive aquaculture practices, factors such as high stocking density, frequent handling, and temperature fluctuations often cause skin injuries, compromising the integrity of this protective barrier and increasing susceptibility to infections. Enhancing skin wound healing is thus critical for fish health and aquaculture productivity.

Zebrafish (*Danio rerio*) have become a widely recognized model for studying vertebrate skin regeneration due to their rapid wound closure ability. Recently, a new laser ablation method has provided precise and reproducible control over wound size and depth, further enhancing experimental consistency. The present study utilized the laser ablation model in adult zebrafish to evaluate the efficacy of phytobiotic-based dietary additives in accelerating wound healing and promoting efficient skin regeneration.

## Material and methods

Adult wild-type zebrafish were maintained at 28 °C in 10 L tanks with a 14-hour light/10-hour dark cycle and fed twice daily. Fish were randomly allocated into experimental groups (n=10 per group), receiving either phytobiotic-based dietary additives or PBS control by oral gavage at 500 mg/kg, administered twice weekly for 14 days before injury. Full-thickness skin wounds (~2 mm diameter) were induced using a 3 W laser on the left flank under anesthesia. Following laser ablation, fish were housed individually in aerated 3 L tanks with water renewal every two days, and dietary treatments continued until the trial conclusion. Wound areas were photographed at days 5, 10, 15, 20, 25, 30, and 35 post-ablation and analyzed using ImageJ software, expressed as the wound-closure percentage (WC%). At 10 days post-injury, skin samples were collected, embedded in paraffin, sectioned, and subjected to hematoxylin-eosin (H&E) staining and cytokeratin 18 (CK18) immunostaining to assess re-epithelialization and tissue remodeling activity.

**Figure 1.** Zebrafish skin wound healing assay using laser ablation. A laser was used to create precise skin wounds, and healing was compared between control and treatment groups to assess the effect of dietary phytobiotics.




## Results

Following laser ablation, mortality rates remained low across all treatments and control groups, confirming minimal stress induced by laser ablation. Among the tested additives, APEX (Adisseo), a phytobiotic-based formulation containing a mixture of medium-chain fatty acids and essential oils, significantly accelerated wound healing. Fish treated with the additive achieved 25% wound closure by 9.3 days and 50% closure by 16.2 days, substantially faster than the control group (26.7 days), representing nearly a 40% improvement in skin recovery. Histological assessments and CK18 immunostaining further confirmed enhanced re-epithelialization and tissue remodeling in APEX-treated fish. These findings indicate that dietary phytobiotic effectively stimulate both early and late phases of wound repair. Overall, the finding demonstrates strong potential as a dietary strategy to improve skin resilience and accelerate regeneration processes in fish, potentially reducing dependence on chemical treatments.

# LEVERAGING IMAGE SEGMENTATION MODELS: APPLICATION OF THE SEGMENT ANYTHING MODEL (SAM) IN FISH BEHAVIOUR RESEARCH IN RAS

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## Introduction

Automatic monitoring of farmed fish can lead to improvements on feeding management and a better understanding of stress events. We therefore present an algorithmic pipeline to leverage the SAM[1] model to cut out fish segmentations without the need for costly fish annotations. We also realized the downstream tasks of calculating orientation angles and identifying fish that do not swim in similar orientations as their close conspecifics.

## Materials and methods

A top camera with a video resolution of 1080p @ 30fps was installed above each octagon-like tank (6 tanks, volume of 3.3m$^3$ each). A total of 1000 Atlantic salmon with a weight of 40 grams ± 10 grams were placed in each tank. Using segmentation masks from SAM[1] we calculated the orientation of detected fish in video frames. Then we determine if those fish are swimming coherently with its peers by filtering out fish in each k-mean cluster (which used the spatial coordinates of fish) that fell out of the interval with the median absolute distance around the circular median angle of the cluster, respectively. Aggregating the results gave rise to abnormalities scores.

## Results and Discussion

We were able to show that both feeding and panic avoidance could be measured with these three metrics. We also showed that panic avoidance has a stronger spike/fall, meaning the intensity of reaction and the increase/decrease in our metrics was proportional. Further work is needed to differentiate between different behavioral patterns that could occlude results. Additionally, the detection count could be increased in the future to reflect the tank behavior more reliably. Overall, the presented methodology tries to push towards easily accessible and interpretable readings for facility personnel.

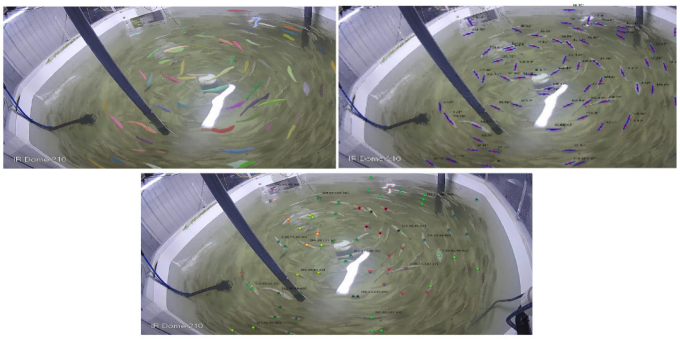

*Figure 1 The algorithmic pipeline from left to right: Segmentation masks (upper left) are used to calculate orientation angles (upper right) which then are used for analysis whether fish are swimming in similar orientations as conspecifics that are nearby (fish in the same cluster have the same dot color). A red triangle indicates that the fish individual is swimming with different orientation than its close peers, a green triangle indicates similar swimming orientation (bottom).*

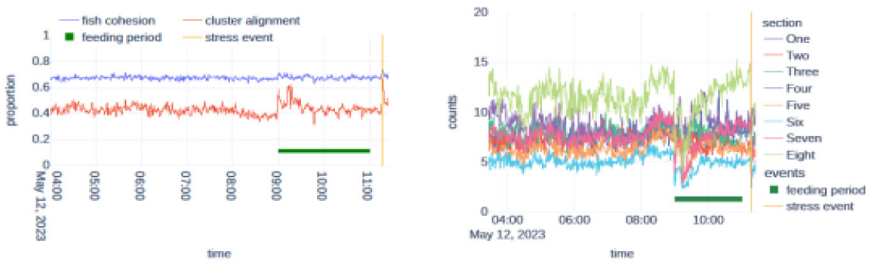


*Figure 2. Fish cohesion and cluster alignment as abnormality scores (left) and the detection counts by tank section (right). The green horizontal line indicates the feeding period, and the vertical orange line the stress event at 11:17.*

**Acknowledgments**
This work was funded by the Norwegian Research Council, Project number 320717 and 323300.

1. Kirillov et al. Segment Anything. arXiv:2304.02643, 2023.

# CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF IMMUNOGLOBULIN T (IGT) UPON VIRAL INFECTION AND TEMPERATURE CHANGES IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Fish inhabit in aquatic environment which is full of pathogens which may cause diseases outbreak. Therefore, fish mucosal immune system, including the mucosal associated lymphoid tissues (MALTs) and immunoglobulins (Igs) serve as the first line of defense in protecting fish from the infection of aquatic pathogens. Grouper which is one of the most economically important species in Taiwan, has been threatened by the nervous necrosis virus (NNV) which can cause almost 100% death for decades. To investigate the grouper mucosal immune system in against to NNV infection, the orange-spotted grouper immunoglobulin T (osgIgT) was cloned and characterized in this study. Real-time quantitative PCR (qPCR) results revealed that osgIgT was highly expressed in grouper MALTs. Moreover, the osgIgT was significantly responsive to the immune stimulants lipopolysaccharide and poly I:C, which mimic the bacterial and RNA viral challenges respectively. Upon NNV infection, the osgIgT transcripts was found upregulated in MALTs, and immunofluorescence analysis demonstrated colocalization of osgIgT with the NNV particles, suggesting a direct role of mucosal immunity against viral infection. Besides, the grouper was treated with different temperatures to investigate the expression of osgIgT upon thermal stresses and the results showed that osgIgT is more responsive to low temperature treatment. In conclusion, this study evaluates the important role of osgIgT in the MALTs of grouper upon viral infection and temperature modulation. These information may contribute to the development of disease management strategies in marine aquaculture.

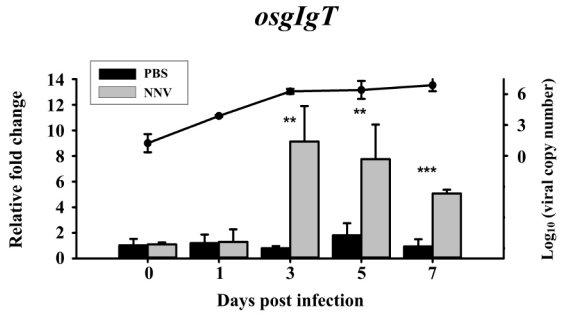


Fig 1. The transcript expression of *osgIgT* in NNV infected orange-spotted grouper larvae at different timepoints were analyzed using qPCR, using *β-actin* as housekeeping gene. The data represented means and standard errors of six fish. Values were compared using the Student's t-test (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

# ACUTE TOXICITY, BIOACCUMULATION AND ELIMINATION OF PROMETRYN IN TILAPIA *Oreochromis niloticus*

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Tilapia juvenile were aqueous exposed to different concentrations of the herbicide prometryn to investigate its acute toxicity, bioaccumulation and uptake and elimination rates. First, a 96-h acute toxicity test was carried out. The resulting 96 h $LC_{50}$ was 5.49 mg/L, and the 96 h $LC_{10}$ was 5.02 mg/L. Then, fish were exposed to 0.55 mg/L (1/10 96 h $LC_{50}$) and 0.055 mg/L (1/100 96 h $LC_{50}$) of prometryn solution for 28 days, followed by 14 days of elimination in clean groundwater.

The accumulated concentration levels in various tissues were always higher in the high concentration compared to the low concentration. The highest accumulated concentration of prometryn in various tissues in the 0.055 mg/L treatment were for muscle: 0.136 ± 0.0616 mg/kg (1 d), liver: 3.74 ± 2.95 mg/kg (7 d), gill: 0.971 ± 1.45 mg/kg (1 d) and blood: 0.0716 ± 0.0669 mg/kg (22 d). In the 0.55 mg/L treatment, the highest levels were for muscle: 1.27 ± 0.284 mg/kg (1 d), liver: 16.9 ± 12.7 mg/kg (7 d), gill: 8.11 ± 3.02 mg/kg (1 d) and blood: 0.751 ± 0.0775 mg/kg (22 d). The highest bioconcentration factor (BCF) of 93.1 was observed in the liver when exposed to the low concentration. Besides, for other tissues, the highest BCF were for muscle: 6.03, gill: 32.3 and blood: 2.91, all observed in the 0.55 mg/L treatment. Most of the accumulated prometryn was removed from all tissues within 24 h after the organisms were transferred to clean water. However, management of using prometryn in China aquaculture should be improved to prevent possible ecotoxicological effects and ensure food safety.

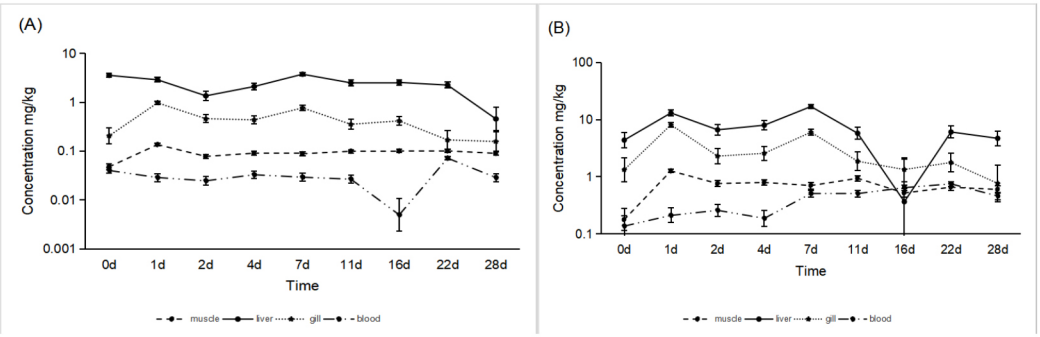


Figure 1. Tissue concentrations of prometryn in the 0.055 mg/L (A) and 0.55 mg/L (B) treatments during the exposure period.

# EFFECTIVE MICROBIAL CONTROL STRATEGIES FOR CONSISTENT HIGH-QUALITY ARTEMIA PRODUCTION

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**Introduction**
Effective microbial control in hatcheries is essential for achieving predictable and consistent outcomes. In production facilities, proactive microbial management, robust biosecurity measures, and thorough cleaning and disinfection protocols create a controlled environment that minimizes the risks of opportunistic bacteria entering or dominating the system.

Our study focuses on microbial management in the live food sector using EG SEP-Art® D-FENSE and a CIP (clean-in-place) agent to enhance biosecurity in the Artemia sector. The CIP agent is used initially to remove bacterial biofilm, reducing the microbial population in the tank. This step aids D-FENSE technology in controlling bacterial growth in the hatching and enrichment tank, ensuring consistent production of high-quality live food both nutritionally and microbially.

**Materials and methods**
Artemia cysts were hatched for 20 hours at a temperature of 28±1°C, and a salinity of 38ppt. The pH of the hatching medium was maintained above 8.2 during the complete hatching cycle using 120ppm of NaOH (30%) as needed. Two treatments were tested:

1) EG SEP-Art® (INVE Aquaculture, Belgium)
2) EG SEP-Art® D-FENSE (INVE Aquaculture, Belgium)

After hatching, Artemia nauplii were harvested, rinsed, and restocked for 22-hour enrichment using Easy DRY SELCO (INVE Aquaculture, Belgium) in two 360 ppm doses, one at the start and another after 10 hours. Temperature was maintained at 27±1°C, pH above 7.5 with 0.5 $g.L^{-1}$ bicarbonate initially and 120 ppm NaOH as needed. Dissolved oxygen was kept above 100% using pure oxygen. Three treatments were tested:

A) Traditional enrichment using rinsed Artemia nauplii from EG SEP-Art® cysts without applying a CIP agent during enrichment (Tr A).
B) Traditional enrichment using rinsed Artemia nauplii from EG SEP-Art® D-FENSE cysts with CIP agent (Tr B): 150 ppm at the start (T24+0) and 150 ppm after 10 hours (T24+10).
C) All-in-one enrichment using Artemia from EG SEP-Art® D-FENSE cysts in the same tank with CIP agent (Tr C): 150 ppm added after 18 hours (T18) and another 150 ppm after 28 hours (T28).

The microbial quality of the hatching and enrichment medium was assessed using traditional plating methods on TSA (1.5% salt) and TCBS agar. Water samples were filtered to remove nauplii. The treatment's effect on the enrichment process was evaluated by FAME (Fatty Acid Methyl Ester) analysis of the nauplii at the trial's end.

**Results**
Using EG SEP-Art® D-FENSE cysts significantly enhances the efficient production of high-quality live food. Results showed a 3-log reduction in total bacterial load and a 4-log reduction in Vibrio load in the hatching medium 24 hours post-hatching.

The use of EG SEP-Art® D-FENSE cysts during hatching, combined with the application of the CIP agent during traditional enrichment practices (Tr B), resulted in a 3-log reduction in both Vibrio and total bacterial load compared to the control treatment (Tr A).

In the all-in-one enrichment procedure, bacterial levels remained similar to Tr B, indicating that EG SEP-Art® D-FENSE with the CIP agent effectively controls microbial development. This method eliminates the labor-intensive step of harvesting and cleaning from traditional practices.

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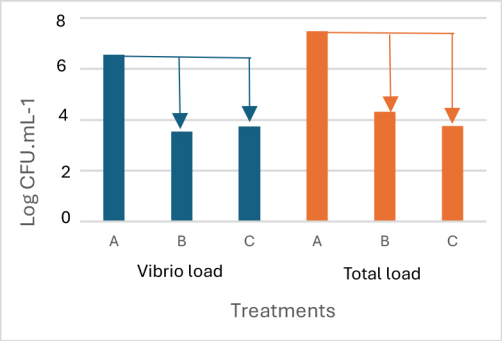


Figure 1. Vibrio load and Total bacterial load found in the enrichment medium after 24+22h & 40h for respectively the traditional way of enrichment (TrA and TrB) and the all-in-one procedure (Tr C).

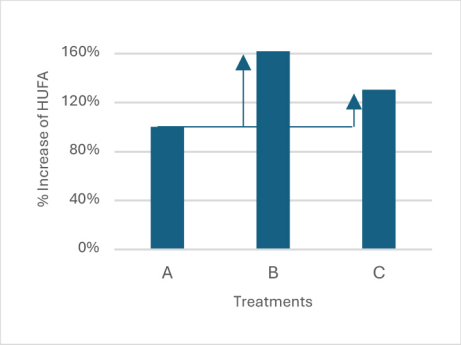


Figure 2. % of Increase in the total HUFA in Tr. B and Tr. C compared to Tr. A

Enhancing water quality with the CIP agent significantly improved enriched Artemia nauplii. HUFA content increased by 60% in Tr B and 30% in Tr C compared to Tr A (Figure 2). Lower bacterial levels likely reduce HUFA utilization and conversion. Improved water quality correlates with increased vitality, better water filtration, and enhanced nutrient uptake, thereby enhancing nutritional value.

**Conclusions**

These results demonstrate the effectiveness of combining EG SEP-Art® D-FENSE with the selected CIP agent in improving the microbiological and nutritional quality of Artemia. The study highlights the importance of strong biosecurity measures to enhance and standardize live food production.

Using EG SEP-Art® D-FENSE cysts with CIP agents (all-in-one treatment) streamlines hatchery operations by eliminating the intermediate harvest and rinsing step, reducing labor while maintaining high nutritional and microbial quality standards.

# UNVEILING ANTIVIRAL POTENTIAL OF PLANT EXTRACTS AGAINST VHSV IN RAINBOW TROUT

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Aquaculture has emerged as a key solution to meeting the world's demand for fish feed over the last few decades. However, the sector faces significant economic challenges due to disease outbreaks. While chemical agents are commonly employed for disease management, their potential ecological impacts raise concerns. Plant extracts, with their inherent pharmacological properties, present a promising alternative. This study investigated the *in vitro and in vivo* antiviral activity of four plant extracts – *Vitis vinifera* (grapevine), *Rosmarinus officinalis* (rosemary), *Glycyrrhiza glabra* (licorice), and *Punica granatum* (pomegranate) – against viral hemorrhagic septicemia virus (VHSV) in rainbow trout. These plant species were selected based on existing evidence of their immunostimulatory or antiviral properties, with the aim of identifying novel antiviral compounds for aquaculture applications. The antiviral activity of plant extracts against VHSV was initially evaluated *in vitro* using the EPC cell line. Cytotoxicity assays established the working concentrations used in subsequent experiments. Then, the antiviral activity of the plant extracts, before or during the course of VHSV infection, were assessed and viral loads were evaluated by means of a focus forming units assay. *In vitro* results demonstrated that all tested plant extracts exhibited antiviral activity, reducing viral load by over 50% in all cases. Subsequently, an *in vivo* study was carried out on rainbow trout to evaluate the use of feed supplemented with plant extracts, analysing the immune response by RT-PCR and toxicity by haematological and biochemical techniques. The *in vivo* antiviral activity was further evaluated by challenging rainbow trout with VHSV and then feeding them with a diet supplemented with plant extracts and monitoring their survival for 20 days. The resulting survival rates exhibited variability depending on the specific plant extract administered, with certain extracts demonstrating superior outcomes. These findings suggest that the use of these plant extracts offers a potential alternative for controlling viral diseases in aquaculture.

# A SHADOW TWIN FOR MUSSEL-SEAWEED INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) IN THE GALICIAN COAST (NW IBERIAN PENINSULA)

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This work focuses on the integrated culture of Mediterranean mussels (*Mytilus galloprovincialis*) and sugar kelps (*Saccharina latissima*) in the Ría de Ares-Betanzos (Galicia, NW Iberian coast), where the latter are cultured in longlines within a mussel raft polygon. Both seaweeds and mussels are low-trophic species which feed, respectively, uptaking seawater dissolved and particulate nutrients and, consequently, their culture does not imply any external food supply. For this reason, seaweed and bivalve aquaculture are among the most sustainable seafood production systems. However, they are also strongly dependent on local environmental conditions making them particularly vulnerable to environmental variability and associated risks such as extreme weather conditions and climate change.

In this context, the development of digital tools to model the growth of the species involved in the integrated culture system under variable environmental conditions is crucial, not only to evaluate and optimize the performance of implemented systems, but also as a site selection tool for the implantation of new mussel-seaweed farms.

We propose an integrated model coupling a high-resolution MOHID model, an individual-based net-production dynamic energy budget (DEB) model for mussels, and an individual based model (IBM) for seaweeds. The MOHID model, incorporates the effect of rafts and longlines in surface currents. The mussel DEB model, which simulates mussel growth under the variable environmental conditions of the culture area, incorporates a carbon-nitrogen (CN) module to estimate carbon, ammonia and nitrate release during growth. The IBM treats each seaweed as a distinct unit, with its own set of physiological and structural variables - such as biomass, nutrient content, and growth rate. These individual seaweeds interact dynamically with the local environment, responding to seawater conditions such as light availability, temperature, and salinity, as well as nutrients, including those released by the nearby mussel rafts in integrated aquaculture systems. This allows for a realistic simulation of heterogeneous growth patterns and nutrient cycling.

The proposed shadow twin can be used to test different culture strategies (seeding and harvest schedule, seeding density, spatial longline-raft configuration) under the variable environmental conditions of the culture area. Consequently, given the strong dependence of offshore IMTA on spatial planning and integrated coastal management, we provide a valuable asset to support producers and policymakers for the development of sustainable IMTA systems.

# ENVIRONMENTAL ENRICHMENT IMPROVES WELFARE OF JUVENILE ATLANTIC SALMON (*Salmo salar*)

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**Introduction**

The breeding of animals for research purposes must meet high standards of animal welfare to ensure that experimental outcomes accurately reflect their biology. In fish, enriched environments (EE) support the expression of natural behaviours and promote positive animal welfare (PAW) (Jones et al., 2021). This is essential not only for obtaining valid and reproducible results but also for developing improved husbandry practices.

In Atlantic salmon (*Salmo salar*), early-life exposure to EE has been associated with increased brain plasticity and enhanced cognitive (Salvanes et al., 2013) function. In contrast, barren environments often lead to maladaptive behaviours and cognitive impairments, negatively impacting welfare and potentially compromising experimental data.

Previous studies have shown that EE improves the ability of fish to cope with stress and reduces the activation of the endocrine stress response. These benefits are linked to increased neuronal proliferation and behavioural flexibility, both key components of welfare. Serotonin (5HT) plays a central role in regulating anxiety, aggression, depression, and the integrated stress response. It also regulates neurogenesis and synaptic plasticity, both essential for learning, memory, and emotional regulation. Dysfunction in this system can quickly impair these functions (Backström & Winberg, 2017).

This study tested the hypothesis that early-life exposure to EE enhances stress resilience in juvenile Atlantic salmon by modulating serotonergic activity.

**Methods**

Atlantic salmon fry was randomly allocated into eight 700 litre tanks (n = 375 per tank, initial weight: 1 g), with a continuous water flow (900 l/h), 24-hour light regime, and temperature maintained at 12 °C. Four tanks were provided with structural EE consisting of a circular PVC grid (70 cm diameter) covered with artificial plastic plants, while the remaining four tanks served as non-enriched (NE) controls. Fish were kept under these conditions for 12 weeks.

Throughout the experiment, fish behaviour was monitored and novel object tests were conducted to assess emotional state. Three days before and 30 minutes after an acute stressor (5-minute chasing), 10 fish from each tank were euthanized using an MS222 overdose. Brain and blood samples were collected to analyse cortisol levels, brain serotonin content and absolute gene expression for markers related to stress, cognition, and serotonergic function.

**Results and discussion**

Fish reared in enriched conditions exhibited greater group cohesion, reduced neophobia, and more stable serotonin levels compared to NE fish. These results suggest that EE helps mitigate the effects of stress and promotes more positive emotional states.

The highest levels of serotonin (5HT) and its primary oxidative metabolite 5HIAA were found in the EE group. Following the acute stressor, significant increases were observed in 5HIAA levels and the 5HIAA/5HT ratio, indicating elevated serotonergic turnover. However, only NE fish showed a significant drop in 5HT levels post-stress, suggesting that EE fish maintained more stable serotonergic tone and were better equipped to cope with the stressor.

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Gene expression analysis revealed increased levels of markers associated with neuronal development and activity (*bdnf*, *ndf1*), cellular stress (*hsp90*, *hsp70*), and serotonin synthesis (*tph2*) in enriched fish after the stress event. Strong correlations between *bdnf* and both *hsp90* and *tph2* further support a link between neural plasticity and the stress response.

Overall, our findings demonstrate that early-life EE enhances cognitive function, stress resilience, and emotional state in juvenile Atlantic salmon. These outcomes highlight the importance of environmental enrichment in improving PAW in captive settings, ensuring that animals are free from negative experiences and have access to positive ones (Prentice et al., 2025).

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# MANAGING WATER QUALITY HAZARDS IN A MODIFIED FLOW- THROUGH AQUACULTURE SYSTEM THROUGH INTEGRATED TREATEMNT METHODS FOR EFFECTIVE FISH PATHOGEN REMOVAL

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## Introduction

Numerous investigations have highlighted frequent large-scale die-offs in olive flounder (*Paralichthys olivaceus*), largely linked to a variety of infectious agents. Key contributors to these episodes include *Miamiensis species*, which trigger scuticociliatosis; *Streptococcus species*, known for causing streptococcosis; viral hemorrhagic septicemia virus (VHSV), an easily transmissible viral invader; *Vibrio strains*, which bring about vibriosis; *Tenacibaculum* bacteria, implicated in gliding motility disorders; the protozoan *Cryptocaryon irritans*, which causes marine white spot illness (MWSD); and *Edwardsiella* bacteria, the source of edwardsiellosis. These organisms pose serious risks to fish farming operations, underlining the need for robust diagnostic systems and intervention measures to curtail disease progression and avoid substantial financial setbacks in the sector.

## Materials and methods

To explore the relationship between bacterial levels, water temperature, and extreme weather at aquaculture sites, a two-year field survey was carried out in Jeollanam-do and Jeju-do, Korea. This study evaluated how environmental and climate variables affect bacterial growth in fish farms. Based on continuous monitoring data, a novel water purification system (TSS-PRS) was developed and installed at a site in Jeollanam-do to test its real-world effectiveness in managing water quality. After deployment, both raw and treated water, along with cultured fish, were analyzed to determine the system's ability to reduce microbial contamination. Detection and quantification of ten critical pathogens were conducted using DNA extraction and real-time PCR techniques to ensure precise microbial assessment.

## Results

In untreated water, bacterial levels rose sharply in Jeollanam-do between July 12 and July 30, 2024—from $10.9\times10^3$ to $5.15\times10^4$ BQV $mL^{-1}$—coinciding with severe weather on July 16, including a peak temperature of 30 °C, 64 mm of rainfall, and wind speeds of up to 8.5 m $s^{-1}$ (Fig. 6e). This surge is likely linked to these meteorological extremes. In contrast, treated water showed a steadier pattern, with bacterial activity ranging from $3.00\times10^3$ to $7.68\times10^3$ BQV $mL^{-1}$ over the same period. Even during subsequent weather events—such as 45.4 mm of rain on September 21, 26.7 mm on October 22, and winds of 7.9 m $s^{-1}$ on November 26—bacterial levels in both treated and untreated samples stayed below $0.4\times10^3$ BQV $mL^{-1}$, indicating minimal impact.

# GENERAL DETERMINATION OF WELFARE INDICATORS ACROSS THE FISH SPECIES USING THE MONITORING TECHNOLOGIES IN TANKS AND CAGES

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## Introduction

Fish welfare is increasingly recognized as crucial for sustainable aquaculture, and minimizing stress is a key aspect of good welfare. New monitoring technologies are making it possible to observe fish behavior and well-being more closely than ever before. This collaborative study – involving experts from USB (Czech Republic) HCMR (Greece), SINTEF and NOFIMA (Norway), and CSIC (Spain) – explored how emerging tools like underwater video cameras, echosounders (sonar), and electronic biosensor tags can be used to monitor fish welfare. The aim was to identify common welfare indicators by detecting stress responses across multiple key European aquacultural fish species (Atlantic salmon, European seabass, and gilthead seabream) in different aquaculture systems (land-based tanks and net pens). By uniting efforts across institutions and species, the project provides a broad, high-level perspective on using technology to track fish well-being in real time.

## Materials and methods

Experiments were carried out in both controlled tank environments and marine open net pens to reflect common aquaculture settings. At each site, the team employed a multi-technology approach to monitoring the fish. Underwater video cameras were used to record fish behavior and schooling patterns, while an acoustic echosounder was deployed in the large net pen setups to track how the fish school distributed themselves in the water column. In the tank trials, some fish were fitted with data-logging tags (including acceleration sensors) that recorded their movement and activity levels. All three species (salmon, seabass, and seabream) were subjected to a mild, short-term stress challenge designed to mimic routine farm events – most often a crowding of the fish into a smaller space, and in the case of the net pens, the additional disturbance of a net cleaning procedure. These stressors were applied in a harmonized way across the different experiments so that results could be compared between species and environments. The combination of video, sonar, and tagging at each experimental site (as available) allowed researchers to capture complementary data streams and observe how each technology detected changes in the fishes' condition during and after the induced stress.

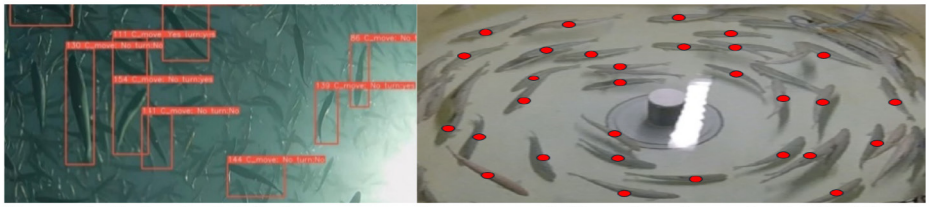

*Figure 1. Individual fish detection in the net pen (left) and in the tanks (right. The short tracks of the fish position serve as an individual fish behaviour parameter. Group-based behaviour parameters were then calculated from the individual-based parameters.*

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## Results

The monitoring tools successfully detected behavioral changes in the fish when a stress event occurred, although the specific responses varied by species and context. In general, when the fish were crowded or otherwise disturbed, the video recordings revealed noticeable shifts in behaviour – for example, altered swimming speeds, tighter schooling behaviour, or changes in preferred depth or location in the tank/net pen. The echosounder data from the salmon net pen similarly showed changes in how the school was distributed, such as fish moving away from the area of the net pen being cleaned, indicating avoidance behaviour. The sensor tags on individual fish in the tank trials captured spikes in physical activity corresponding to the moment of stress, confirming that those fish were reacting even if it was not immediately obvious on camera. Notably, all three species (salmon, seabass, and seabream) exhibited some form of stress response that these technologies picked up, but the magnitude and form of the response were species- and environment-dependent. For instance, the salmon in a net pen did not show as dramatic a change during crowding as salmon in a confined tank or as other species did, though even in the net pen subtle indicators (like slight increases in swimming activity under certain conditions) were observed. These findings highlight that species may display stress in different ways in different settings, underlining the importance of interpreting welfare indicators in context. Importantly, by using multiple methods together, the study was able to detect these varied responses more reliably – one method often confirmed or supplemented the observations of another, giving a more complete picture of the fishes' state.

## Conclusion

In summary, this study demonstrates the value of a multi-tech approach to monitoring fish welfare in aquaculture. Modern technologies such as video cameras, echosounders, and active tags each contribute unique insights into fish condition: video provides direct observation of fish behaviors and interactions, sonar offers a broad view of group distribution and movement in large enclosures, and sensor tags yield information on individual fish activity or physiology. These methods are complementary, and an integrated monitoring system that combines them can overcome the limitations of any single tool. For example, acoustic monitoring works well in large, sometimes opaque water environments like net pens, while cameras and tags are especially useful in smaller or clearer systems – using them together ensures that signs of stress do not go undetected. The collaborative, cross-species results of this project suggest that building a cohesive monitoring framework (where camera, sonar, and tagging data are used in concert) will enhance our ability to identify stress responses early and accurately. Such an integrated system could enable farmers and researchers to respond more quickly to welfare issues, ultimately improving fish well-being and health. The study's findings also emphasize that stress indicators are not one-size-fits-all; they depend on the species and context, so a flexible, multi-technology strategy is the most promising route to better fish welfare monitoring across diverse aquaculture conditions.

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# BIG OR SMALL, ONCE OR MORE? OPTIMISING FEEDING FOR REDCLAW CRAYFISH

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**Introduction**

The redclaw crayfish (*Cherax quadricarinatus*) is a freshwater crayfish native to northern Australia and southern Papua New Guinea. It has gained increasing attention in global aquaculture due to its fast growth, high market value, and adaptability to a wide range of environmental conditions.

As a species with omnivorous feeding habits, redclaw crayfish offer flexibility in diet formulation, making them ideal for sustainable aquaculture systems. Optimizing feeding strategies is essential to improve growth performance, feed efficiency, and overall production profitability.

Nutritional research on crustaceans for aquaculture often focuses on feed composition, mostly overlooking the effect of feeding methods. In this study we examine the effect of pellet size and feeding times per day on growth rate and survival of redclaw crayfish in recirculating aquaculture systems.

**Materials and methods**

Crayfish were kept at 27°C in 12 300L flat-bottomed rectangular tanks with a photoperiod of 16h light/8h dark. All tanks were equipped with brush shelters and connected to a RAS system with mechanical and biofiltration.

25 juveniles (1,11g ± 0,58g) were stocked per tank and fed one or four times per day with 2 or 6mm sized pellets (Carp EX, Aquabio) for 12 weeks. Restricted feeding was applied and daily feeding level evolved from 5% of total biomass (week 1-3) over 4% (week 4-6) to 3% (week 7-12). Each treatment was performed in triplicate.

Every three weeks, all live animals were counted and weighted. Survival percentage and Specific Growth Rate (SGR) were calculated.

**Results**

Specific Growth Rate is determined as the Average Body Weight increase percentage per day between stocking and the final sampling on week 12.

SGR was the highest in the tanks fed 4x per day with 2mm pellets ($2.37\%d^{-1} \pm 0.03\%d^{-1}$) and the lowest in the tanks fed 1x per day with 6mm pellets (2.11 %d-1 ± 0.02 %d-1). A Kruskal-Wallis test followed by post hoc Dunn's tests with Bonferroni correction only shows a significant difference between treatment 4x 2mm and 1x 6mm. However, the SGR in the tanks fed 1x per day with 2mm pellets and 4x per day with 6mm pellets also seem relatively high compared to the 1x 6mm treatment.

The dot plot below (left) shows the SGR for each tank of the four treatments on week 12.
Survival is determined as the percentage of animals per tank that survive between stocking and the final sampling on week 12.

Survival was the highest in the tanks fed 1x per day with 6mm pellets (76.00% ± 10.58%) and the lowest in the tanks fed 1x per day with 2mm pellets (60.00% ± 8.00%). However, no significant differences between treatments could be detected.

The dot plot below (right) shows the Survival for each tank of the four treatments on week 12.

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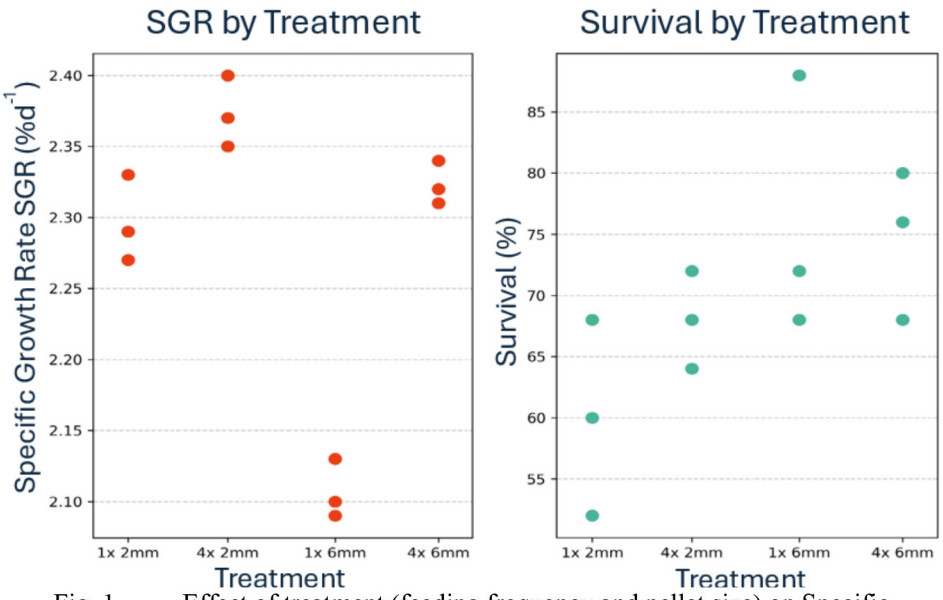


Fig. 1 Effect of treatment (feeding frequency and pellet size) on Specific Growth Rate (left) and Survival (right).

**Discussion**

Feeding four times per day smaller pellets, four times per day larger pellets, or once per day smaller pellets improves growth rate. Crayfish are opportunistic feeders and providing food multiple times may better mimic natural conditions, likely promoting more active and consistent feeding. Dividing the daily ration over multiple feeding times and/or more smaller pellets allows crayfish to digest and assimilate nutrients more efficiently, as they are not overloaded with a single large meal, supporting better growth.

Feeding only once per day larger pellets significantly decrease growth rate. In group settings, dominant individuals may monopolize feed when it is offered only once. A single daily feeding and larger but less pellets decrease the likelihood that subordinate or slower individuals get access to sufficient food, leading to lower average growth rate of the population. Moreover, less feeding times and larger but less pellets may result in less aggressive encounters between animals competing for feed, resulting in a higher survival of slow-growing individuals, again leading to lower average growth rate of the population.

Feeding methods are crucial in redclaw crayfish culture. For juvenile crayfish, multiple feeding events per day are preferred over one, preferrable with relatively small pellets. Although not proven in this experiment, pellet size and feeding frequency may also affect survival and should be adjusted based on the life stage and other culture parameters, such as stocking density and shelter type to reduce aggression and cannibalism, and to optimize production per tank.

# CHANGES TO ZOOXANTHELLAE DENSITY IN *Anemonia viridis* ACCLIMATED TO LOW-LIGHT AQUACULTURE ENVIRONMENTS

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**Introduction**

In Spain, the high demand of snakelocks anemone *(Anemonia viridis*, Forsskål, 1775) for human consumption–along with the invasion of *Rugulopteryx okamurae*–has depleted populations of this species in the Andalusian coast, leading to an indefinite ban on its harvesting since 2023. Aquaculture production of this species has become relevant to help relieve natural populations from exploitation pressure in the region. Because they harbor photosynthetic dinoflagellates in their endoderm, *A. viridi*s requires a certain threshold of light intensity in order to perform adequately in aquaculture settings. However, intense solar radiation can induce oxidative stress from UV light exposure and from the increase in intracellular oxygen released by the symbionts (Richier et al., 2005). Moderating the amount of received light intensity could improve the performance of the animals in aquaculture settings (Coll et al. 2025).

Histological assessment can help detection of acclimation responses to different light conditions, which then can complement assessment of the physiological state of the animals (Coll et al., 2025). The goal of this study was to (1) characterize the histological structure of *A. viridis* body wall, and (2) study histological alterations of this structure associated to different light environments in aquaculture settings.

**Materials and Methods**

140 wild individuals of *A. viridis*, previously acclimated, were randomly distributed into two identical outdoor recirculation tanks (8 $m^3$) with three partitions each. Both tanks were exposed to natural photoperiod (12:12, L:D) and maintained at 21 ± 2 ºC, but one of them had a mesh cover that blocked approximately 99% of incident illuminance. After four weeks, tentacles from three animals per tank were dissected and fixated in Bouin's solution for 48 hours. Following histological processing, 5 µm longitudinal tentacle sections were stained with Masson-Goldner's trichrome. Finally, section micrographs were analysed with Image J to measure histological layers thickness, as well as density of symbiont cells per $\mu m^2$ of endoderm. Mean values were compared for control and shaded partitions using a t-test with a significance of $\alpha = 0.05$. Statistical analysis was performed with R 4.3.

**Results and Discussion**

*A. viridis* display the typical tri-layered structure found in cnidarians, composed of two tissular layers (ectoderm and endoderm) separated by extracellular matrix (mesoglea) (Figure 1). The outer ectoderm hosts an elevated number of cnidocytes of different types: spirocysts (adhesion-specialized cells with a coiled filament) stained brightly with acid fuchsine, while nematocyst (stinging cells with a straight filament) appeared stained with hematoxylin. The mesoglea, a collagen-rich extracellular matrix, stained intensely with anillin blue and displayed several cell nuclei belonging to amoebocytes (immune effector cells). Finally, the inner endoderm is dominated by symbiont cells, and features occasional secretory cells. Masson-Goldner trichrome was an effective staining technique to differentiate cnidarian body wall tissular and cellular components.

Comparison of each of the three layers' (ectoderm, mesoglea and endoderm) thickness revealed no significant differences between anemones kept at control conditions and in a shaded setup ($p = 0.412$, $p = 0.118$ and $p = 0.43$, respectively) (Figure 2), although there was a slight pattern of thicker mesoglea for shaded samples. However, symbiont cell density was found to be significantly higher in the shaded setup ($p < 0.05$). significantly higher in the shaded setup ($p < 0.05$).

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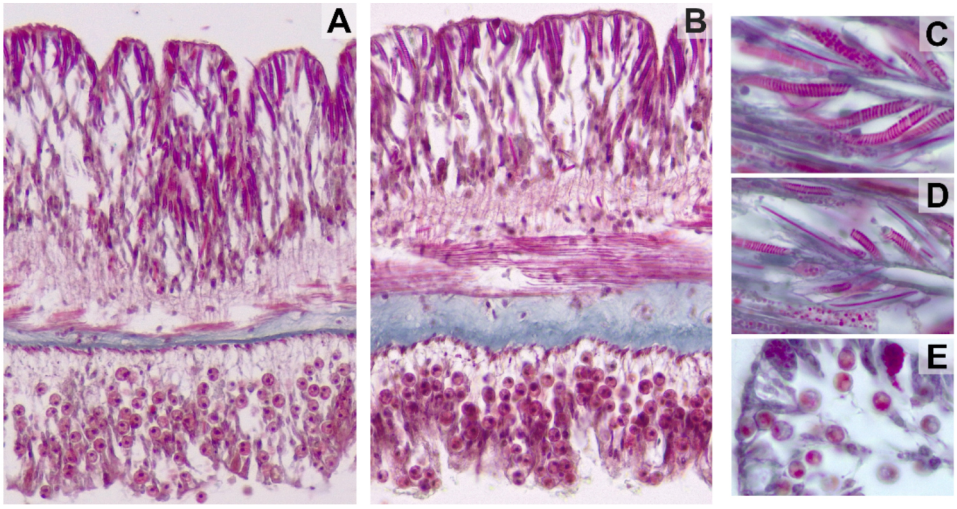


***Figure 1.*** Comparison of the tentacle structure of *A. viridis* in control (A) and shaded (B) conditions (40x). Detail of spyrocists (C), nematocysts (D) and zooxanthellae (E) (100x). Masson-Goldner stain, 5 µm sections.

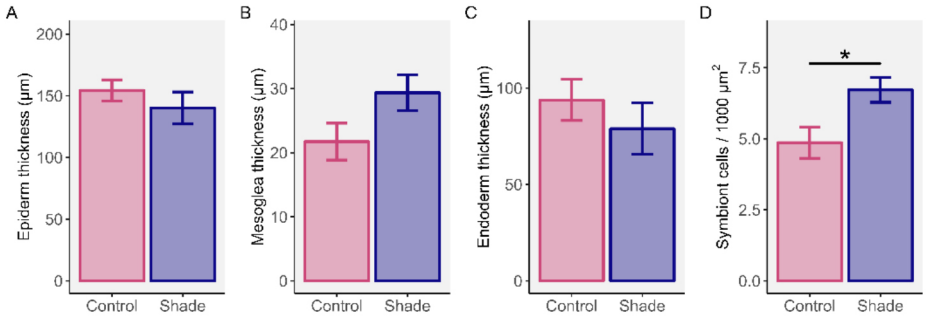


***Figure 2.*** Mean ± Standard Error thickness of epiderm (A), mesoglea (B) and endoderm (C), and symbiont density (D) in control or shade treatments. *: Significative differences between control and shade treatments.

Despite the general lack of histological alterations due to acclimation to different light environments, anemones displayed higher mean density of symbiont cells in their endoderm when acclimated to the shaded setup. This could be part of a compensation response to maintain a similar level of photosynthates flow into the animal host cells. Previous results found no alterations to oxidative status of *A. viridis* in shaded aquaculture conditions (Coll et al., 2025), suggesting that the setup minimized UV exposure while still allowing for sufficient light intensity for photosynthesis. The absence of bleached specimens also supports this hypothesis, but further studies of photosynthetic rates will be necessary to corroborate these results.

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# IMPROVING PIKEPERCH (*Sander lucioperca*) FARMING IN RAS TROUGH POLYCULTURE WITH EUROPEAN CATFISH (*Silurus glanis*)

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## Introduction

This study is part of the POLYRAS[1] project (Interreg GR), which aims to optimize the sustainable production of locally important fish species, such as pikeperch, through polyculture in recirculating aquaculture systems (RAS). Polyculture, a traditional practice in pond aquaculture, aim to exploit species complementarity to improve feed utilization and overall productivity (Thomas et al., 2020). Applied in RAS, polyculture may enhance feed efficiency, increase production, reduce labour and support animal welfare (Kolowski et al., 2014; Thomas et al., 2020; Penka et al., 2021; Amoussou et al., 2022).

This study evaluates, in a recirculating aquaculture system (RAS), whether co-culturing pikeperch (*Sander lucioperca*) with European catfish (*Silurus glanis*), a fast-growing bottom-feeding species, can offer some benefits compared to the monoculture of each species. Specifically, we investigated whether polyculture could maintain or enhance the growth of both pikeperch and catfish, improve overall tank productivity, make better use of uneaten food, and support species welfare. Results related to growth performance and tank productivity are presented in the present paper, while the aspects on feed valorisation and fish welfare will be addressed in future work.

## Material and Methods

The experiment was conducted in 7 x 1 m³ tanks supplied with water at 23°C in a recirculating aquaculture system (RAS) under a 12 h light / 12 h dark photoperiod. Three conditions were tested: pikeperch (*Sander lucioperca*) monoculture, European catfish *(Silurus glanis*) monoculture, and polyculture of both species (ratio 1:1). The initial stocking density was 8 $kg.m^{-3}$. Tanks in monoculture were run in duplicate and those in polyculture in triplicate. Initial mean body weights were 166.6 g for pikeperch and 181.8 g for catfish. Pikeperch were fed with floating pellets (Le Gouessant Percid Grower®, 52% proteins, 15% lipids) at maximal feeding rate, while catfish received sinking pellets (Coppens Silurus Pro®, 46% proteins, 19% lipids) at a restricted rate of 1.5% of biomass. Growth was assessed monthly by individually weighing 20 fish per species and tank, and total biomass was assessed at the start, mid-point, and planned end of the experiment. Here, we present preliminary results obtained after 134 days of the planned 174-day experiment. Data were analysed in R. Normality and homogeneity of variances were checked (Shapiro–Wilk and Bartlett tests); ANOVA with Tukey's post-hoc or Kruskal–Wallis with pairwise Wilcoxon tests were applied as appropriate ($P < 0.05$).

## Results

Survival rates were up to 97% for all batches.
For each species, Sspecific growth rates (SGR) were comparable between monoculture and polyculture: 0.77 for pikeperch in monoculture vs. 0.82 in polyculture and 1.22 for catfish in monoculture vs. 1.19 in polyculture. After 134 days, final mean body weights were 467 ± 16g for pikeperch in monoculture and 498 ± 14 g in polyculture; and 937 ± 35 g for catfish in monoculture and 897 ± 28 g in polyculture, No significant differences were detected between rearing conditions for either species ($P > 0.05$).

Food conversion ratio (FCR) and final biomass in polyculture were intermediate between those observed in both monocultures. FCR values were 1.05 for pikeperch monoculture, 0.92 for polyculture and 0.82 for catfish monoculture. At 134 days, mean total biomass per tank was 22.1 ± 1.8 kg for pikeperch monoculture, 31.3 ± 2.4 kg for polyculture and 41.2 ± 6.4 kg for catfish monoculture. No significant differences were found between conditions ($P > 0.05$).

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**Conclusions and perspectives**

Current results indicate that pikeperch–catfish polyculture in RAS can sustain favorable growth performance without compromising survival of both species. The last 40 days of production will be critical to confirm these performances and to validate the system's potential for enhancing feed utilization while maintaining fish welfare. To further evaluate the potential of the system, isotopic analyses will be used to identify ingested feed and confirm the hypothesis of the valorization of uneaten feed. For the assessment of fish welfare, we will examine fin condition in pikeperch and fin and barbels in European catfish, as well as (adapted from Policar et al., 2016). Additionally, cortisol levels in pikeperch scales will be measured to monitor physiological stress (adapted from Carbajal et al., 2018).

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1. Optimisation de la production aquacole en RAS (système d'aquaculture recirculé) et diminution de son empreinte écologique par la polyculture dans la Grande Région

# CORRELATION BETWEEN SHELF-LIFE AND TOTAL VOLATILE BASIC NITROGEN (TVB-N) CONCENTRATION IN FARMED SEA BASS AND SEA BREAM STORED UNDER VACUUM (VP) OR IN MODIFIED ATMOSPHERE PACKAGING (MAP) AT 4 ± 2 °C

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## Introduction

Fish meat is very perishable due to indigenous and microbial enzymes, which play a key role in spoilage and shelf life. The deterioration processes are rapid, progressive and strongly influenced by rearing, harvesting, slaughtering, handling, and storage conditions. Usually, the shelf life of gutted fish VP and MAP packaging stored at 4 ± 2 °C is approximately 9 days. The spoilage includes the production of short-chain peptides, amino acids and other non-protein nitrogen molecules, trimethylamine, TVB-N, sulfur compounds, lactic acid, acetic acid, ethanol, formaldehyde by tissue demethylase and formic acid, ketones and esters by bacterial enzymes. The aim of this work was to correlate the shelf life to TVB-N, concentrations to propose limits of sensory acceptability of farmed gutted sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) stored at 4 ± 2 °C.

## Materials and methods

Samples consisted of 3 different lots of farmed sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). The sea bass (474–578 g, 35 cm long), and the sea bream (404–440 g, 25 cm long) were bred in sea cages, collected, and slaughtered after a bath in water and ice. The fish were VP (-1.0 bar) and MAP (80% $N_2$, <1% $O_2$, 20% $CO_2$) by an Orved VM53 vacuum machine (Musile di Piave, Italy) and stored at 4 ± 2 °C until analysis. The packaging was a multilayer film consisting of polyethylene terephthalate (PET) with a thickness of 12 µm, aluminum with a layer thickness of 9 µm, nylon with a thickness of 15 µm, and polyethylene (PE) with a thickness of 75 µm. On day of sampling, three samples were collected and subjected to physico-chemical analysis (TVB-N, formaldehyde and formic acid; 1,2). Additional samples were subjected to sensorial analysis. Data were analysed using Statistica software version 7.0 (Statsoft Inc., Tulsa, OK, USA). The values of the different parameters were compared by a one-way analysis of variance, and the means were then compared using the Tukey's honest significance test. Differences were considered significant at $p < 0.05$. Sensorial analyses were performed by 20 non-professional and non-trained assessors (10 women and 10 men, representing food technology students and typical consumers). Sensory analysis was performed based on the triangle test (1). Three lots of 10 sea bass and sea bream samples, were evaluated by tasters who were asked to evaluate the influence of time 0 versus 15 days storage. In short, three samples, wrapped in aluminum foil, two of which were identical, coded with three-digit numbers, were given in a randomized order in a quiet room, and the assessors were asked to identify the different samples and their responses were collected on a paper card. The assessors were eventually required to provide an answer. Those who were able to distinguish between the samples were then asked to indicate which sample they preferred and/or whether both samples were acceptable. The statistical evaluation of the results was carried out according to ISO (3) and Stone and Sidel (4).

## Results

TVB-N concentration changed during storage ($p < 0.05$). At the beginning of storage, the TVB-N value of the tested gutted sea bass and sea bream was less than 14.0 mg N/100 g and then increased reaching values of 41.9 ± 0.2 and 41.5 ± 0.2 mg N/100 g, respectively (Table 1,2). These values indicate that a significative spoilage started some days before 15 days. Indeed, during 12 days of storage, TVB-N values were less than 34.0 mg N/100 g in both fish species; this value is considered acceptable according to the limit proposed by EC/1995 (5), which is 35 mg N/100 g. The level of formaldehyde, as expected, increased till 6 days of storage, due to the endogenous enzymatic activity ($p < 0.05$). Then it decreased as it was degraded to formic acid by tissue enzymes. Indeed, the concentration of the formic acid increased in both the fish species during all the storage up to 15 days ($p < 0.05$). However, both the formaldehyde and formic acid did not influence the acceptability of the fish because they were less than 20 mg/kg. No significative differences ($p > 0.05$) were observed between the two types of packaging.

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**Table 1: Physico-chemical evolution in gutted sea bass**

| Sea Bass | | | | | | |
|---|---|---|---|---|---|---|
| **Days** | **Under vacuum** | | | **MAP (20% $CO_2$; 80% $N_2$; < 0.5% $O_2$)** | | |
| | TVB-N | Formaldehyde | Formic acid | TVB-N | Formaldehyde | Formic acid |
| **0** | $13.0 \pm 0.2^{a}$ | $3.9 \pm 0.1^{a}$ | $1.8 \pm 0.3^{a}$ | $13.5 \pm 0.5^{a}$ | $4.5 \pm 0.3^{a}$ | $2.9 \pm 0.3^{a}$ |
| **6** | $19.9 \pm 0.5^{a}$ | $28.4 \pm 0.4^{b}$ | $3.6 \pm 0.2^{b}$ | $20.1 \pm 0.3^{a}$ | $32.7 \pm 0.3^{b}$ | $4.4 \pm 0.2^{b}$ |
| **9** | $31.5 \pm 1.3^{a}$ | $12.5 \pm 0.2^{c}$ | $5.2 \pm 0.1^{c}$ | $33.2 \pm 0.3^{a}$ | $12.5 \pm 0.5^{c}$ | $5.8 \pm 0.1^{c}$ |
| **12** | $32.2 \pm 0.9^{a}$ | $14.5 \pm 0.7^{d}$ | $5.5 \pm 0.2^{d}$ | $33.4 \pm 0.7^{a}$ | $15.5 \pm 0.2^{d}$ | $6.1 \pm 0.3^{c}$ |
| **15** | $41.9 \pm 0.2^{b}$ | $14.5 \pm 0.1^{d}$ | $5.4 \pm 0.1^{d}$ | $43.3 \pm 0.2^{b}$ | $14.5 \pm 0.7^{d}$ | $5.3 \pm 0.2^{d}$ |

Legend: TVB-N (total volatile basic nitrogen), mg N/100; formaldehyde and formic acid, mg/kg.

**Table 2: Physico-chemical evolution in gutted sea bream**

| Sea Bream | | | | | | |
|---|---|---|---|---|---|---|
| **Days** | **Under vacuum** | | | **MAP (20% $CO_2$; 80% $N_2$; < 0.5% $O_2$)** | | |
| | TVB-N | Formaldehyde | Formic acid | TVB-N | Formaldehyde | Formic acid |
| **0** | $12.9 \pm 0.5^{a}$ | $3.5 \pm 0.1^{a}$ | $2.1 \pm 0.3^{a}$ | $12.7 \pm 0.3^{a}$ | $4.9 \pm 0.4^{a}$ | $3.2 \pm 0.1^{a}$ |
| **6** | $21.9 \pm 0.1^{a}$ | $30.2 \pm 0.2^{b}$ | $4.2 \pm 0.3^{b}$ | $23.5 \pm 1.2^{a}$ | $35.2 \pm 0.1^{b}$ | $6.1 \pm 0.2^{b}$ |
| **9** | $31.5 \pm 1.3^{a}$ | $12.5 \pm 0.1^{c}$ | $5.3 \pm 0.2^{c}$ | $33.2 \pm 0.3^{a}$ | $12.5 \pm 0.3^{c}$ | $5.8 \pm 0.3^{b}$ |
| **12** | $34.6 \pm 0.2^{a}$ | $12.7 \pm 0.2^{c}$ | $5.4 \pm 0.3^{c}$ | $34.0 \pm 0.5^{a}$ | $17.3 \pm 0.2^{d}$ | $7.2 \pm 0.4^{c}$ |
| **15** | $41.5 \pm 0.3^{b}$ | $12.3 \pm 0.3^{c}$ | $5.4 \pm 0.2^{c}$ | $42.8 \pm 0.8^{b}$ | $17.4 \pm 0.1^{d}$ | $7.2 \pm 0.2^{c}$ |

Legend: TVB-N (total volatile basic nitrogen), mg N/100; formaldehyde and formic acid: mg/kg.

Among the non-professional subjects, all the assessors were able to distinguish the different samples by smell, a result that was statistically significant. The samples stored for 15 days were considered unacceptable. Comparing the samples stored for 0 and 12 days, the assessors were required to make a forced choice. Among the non-professional subjects, 7 out 20 assessors recognized the different samples ($p > 0.05$). According to the triangle test, the minimum number of correct responses needed to establish significance at $p < 0.05$ with 20 assessors is 11 (1,2). Despite they recognized the different samples, all the tested samples were considered acceptable. Similarly, the 12 assessors who did not correctly identify the different samples also rated all samples as acceptable.

## Conclusions

Considering the physico-chemical and sensorial analysis of both fish species, a shelf-life limit of 12 days was proposed, corresponding to a total volatile basic nitrogen (TVB-N) level less or equal to 35 mg N/100 g of product.

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# IMPACT OF PHOSPHORUS AND CALCIUM DIETARY LEVELS ON ATLANTIC COD LARVAE PERFORMANCE AND SKELETAL ANOMALIES

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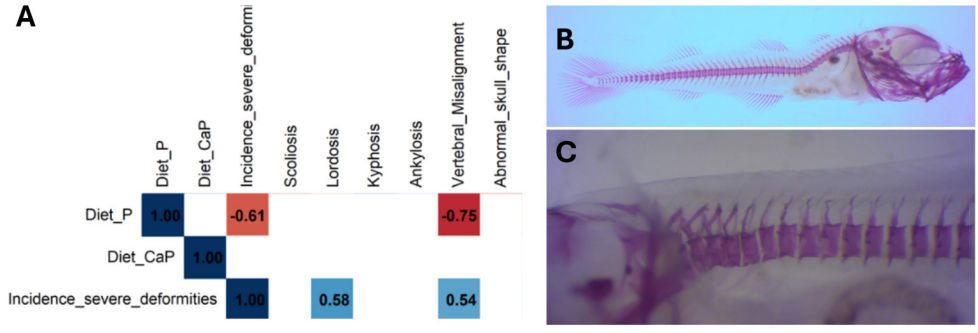
## Introduction

Atlantic cod microdiets need to be optimized, in order to reduce the high variability in growth performance, survival rates and incidence of skeletal deformities, commonly observed in hatcheries. Recent progress has been obtained with the use of alternative live prey in feeding protocols, and with novel microdiet formulations and technology for cod, in recent years. Different factors impact the incidence of skeletal deformities in fish larvae, including water temperature during early development, nutrition, and tank hydrodynamics. Among nutritional factors, phosphorus (P) and calcium (Ca) dietary levels, and their ratio, are usually considered as important to be optimized so to prevent skeletal deformities. The present study evaluated the impact of phosphorus and calcium dietary levels, and their ratio, on larvae performance and skeletal deformities of Atlantic cod.

## Materials and methods

Atlantic cod (*Gadus morhua*) larvae originating from NOFIMA AS (Tromsø, Norway) were randomly distributed in fifteen 200 L tanks at 3 days after hatching (DAH) and kept until 50 DAH (trial end), at the NTNU Sealab facilities (Trondheim, Norway). The tanks were equipped with a self-cleaning system and automated feeders. The light regime was 24h and temperature ranged from 7.1 to 12.3°C. All tanks were fed with the same live feed protocol. Fish were initially fed with enriched rotifers, and using green-water technique. The enrichment used was ENRico (Sparos Lda, Olhão, Portugal), with rotifers being enriched for 8 hours with 2 doses of 0.12 g/million rotifers. To obtain green-water conditions Phytobloom RAS (Necton SA, Olhão, Portugal) was used, by adding 6g/tank/day, in order to keep an algal density of 200000 cell/mL. At day 15 unenriched Artemia (strain AF, INVE, Dendermonde, Belgium) was introduced, and co-feeding of Artemia and microdiets occurred from 15-29 DAH. Enriched Artemia was not used in the present study. The 5 microdiets tested were fed alone from 30 DAH onwards. All diets were produced at SPAROS (Olhão, Portugal) by low-shear extrusion. Two levels of P (1.5 and 2.0%, feed basis) and 3 Ca:P ratios (0.4, 0.6 and 0.8) were tested in the following combinations: P2/0.4; P2/0.6; P2/0.8; P1.5/0.4; P1.5/0.6. Larvae were sampled for growth, Ca and P contents, and skeletal anomalies (staining with alizarin red S). Results were analysed using univariate statistics by fitting linear models, followed by two-way ANOVA (2W-ANOVA) with Type II sum of squares to test for main effects and interactions, as well as principal component analysis (PCA) and correlation analysis.




**A.** Correlation coefficients for significant linear regressions ($p<0.05$).between severe deformities and dietary levels of phosphorus, and Ca:P ratio, over the 15 tanks used in trial. Only significant differences are shown. **B.** Atlantic cod larvae with scoliosis**. C.** Atlantic cod larvae with vertebral misalignment.

*(Continued on next page)*

**Results**

Dietary P and Ca:P (2W-ANOVA, $p>0.38$) had no impact on growth parameters (dry weight, total length) at 30 or 50 DAH ($p>0.65$). Moreover, no interaction was detected between dietary P and Ca:P impact on growth ($p>0.17$). Relative growth rate until 50 DAH was around 9.5%/day. Mortality at 50 DAH was significantly lower ($p=0.03$) at dietary P of 1.5% (6.0%) compared to 2.0% (6.9%). The incidence of severe skeletal anomalies was significantly higher ($p=0.03$) at dietary P of 1.5% (47.24%) compared to 2.0% (25.56%); This lower incidence of severe skeletal anomalies at 2.0% compared to 1.5% dietary P was mostly driven by vertebral misalignment (6.2% vs 17%) and lordosis (4.5% vs 7.%). Other severe anomalies observed were scoliosis (6% average in all treatments), ankylosis (8%), and to a lesser extent abnormal skull shape (1.9%), and kyphosis (0.7%). Dietary P had a highly significant effect ($p<0.002$) on fish content of P, Ca and the Ca:P ratio. Dietary Ca:P ratio had also a significant effect ($p<0.04$) on fish content of Ca and the Ca:P ratio, but not on P contents ($p=0.28$). No significant interactions ($p<0.05$) were observed between Dietary P, and Ca:P ratio, on the mineral contents of Atlantic cod.

**A.** Correlation coefficients for significant linear regressions ($p<0.05$).between severe deformities and dietary levels of phosphorus, and Ca:P ratio, over the 15 tanks used in trial. Only significant differences are shown. **B.** Atlantic cod larvae with scoliosis**. C.** Atlantic cod larvae with vertebral misalignment.

**Conclusions**

Dietary Phosphorus (1.5 to 2% of feed) and Ca:P ratios (0.4 to 0.8) have no impact on growth of Atlantic cod. However, to improve skeletal mineralization, and reduce skeletal anomalies, a dietary Phosphorus level of 2% of feed is recommended. Nevertheless, Ca:P ratio seems to have little impact on skeletal mineralization and skeletal of Atlantic cod. These results highlight the potential of tailoring microdiets for Atlantic cod larvae may bring to improvement of larval and juvenile quality.

**Acknowledgements**

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# FIRST APPROACH TO *Elasmopus rapax* CULTURE (Crustacea, Amphipoda): LIFE HISTORY TRAITS AND HABITAT PREFERENCE UNDER LABORATORY CONDITIONS

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## Introduction

In recent years, amphipods have gained increasing relevance in the fields of aquaculture due to their key role in marine food webs, high reproductive efficiency, and potential as a component of IMTA systems (Fernandez-Gonzalez et al. 2018). Indeed, amphipod feeds might be used in aquaculture to replace *Artemia* in different nursery feeding regimens, as a fishmeal substitute in aquafeeds, or as either live or frozen feed for fish and crustaceans (Herawati et al. 2020, Vargas-Abúndez et al. 2020).

*Elasmopus rapax* is a cosmopolitan marine amphipod found in a wide range of coastal environments, particularly rocky and vegetated bottoms. Its frequent occurrence in fouling communities and tolerance to variable conditions make it a promising candidate for experimental studies.

This study presents a first approach to assessing the feasibility of the culture of *E. rapax* under laboratory conditions, focusing on key biological aspects such as average fecundity and habitat preferences.

## Materials and Methods

Individuals of *E. rapax* were collected from fouling communities of an aquaculture facility located in the western Mediterranean (San Pedro del Pinatar, Murcia). In the laboratory, they were maintained in 10 L aquaria with filtered seawater, controlled temperature (22–25 ± 1 °C) and fed daily with feed pellets and live diet (copepods, Artemia, rotifers and phytoplankton). Two main experiments were conducted:

1. Fecundity: Ovigerous females were selected, and the number of eggs was quantified under a stereomicroscope.
2. Habitat preference: Five types of substrates were offered: (A) artificial algae resembling *Ulva*, (B) plastic bioballs placed on the bottom, (C) ceramic bioballs on the bottom, (D) floating plastic bioballs, and (E) a motorized water filter. The distribution of individuals across the different substrates was recorded after 48h of acclimation and the individuals were counted twice per day for 3 weeks.

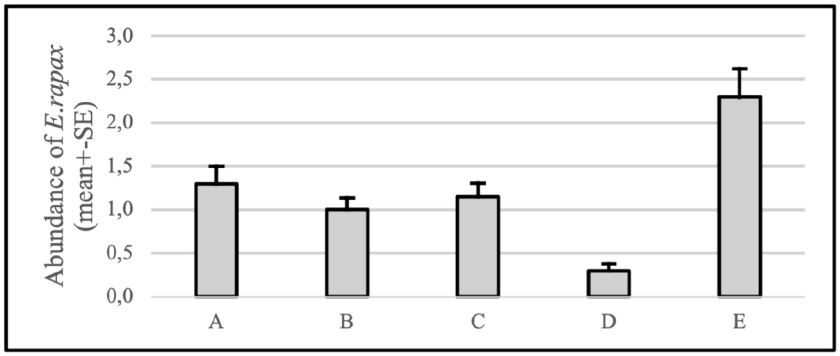


*Fig 1:Amphipods (*E. Rapax individuals*) observed in the different habitats (mean ± SE). (A) artificial algae resembling* Ulva*, (B) plastic bioballs placed on the bottom, (C) ceramic bioballs on the bottom, (D) floating plastic bioballs, and (E) motorized water filter.*

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**Results and Discussion**.
This study is the first reporting a culture system for the amphipod species *E.rapax,* which has been satisfactorily maintained for more than one month. Preliminary fecundity results (11.9 ± 4.3 eggs per female) fall within the range reported for other marine amphipod species, being fecundity often positively correlated with the female's body size (Vargas-Abúndez et al., 2020).

Regarding habitat preferences, the most frequently used substrate was the E (2,29±0,33), followed by A (1,29 ± 0,2) and C (1,15 ± 0,16). B (1,00 ± 0,13). This distribution suggests that structural complexity and substrate are related to algae and rocky habitats and high hydrodynamism, playing a significant role in microhabitat selection for this species.

Based on our results, *E. rapax* is a species with promising potential in aquaculture as a food source for fish and crustaceans due to its manageable nature and possible nutritional value. However, further research is needed to optimize rearing conditions and assess its reproductive and nutritional performance.

**Acknowledgments**
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# STAKEHOLDER ENGAGEMENT AND HEALTH-RELATED METRICS FOR SUSTAINABLE AQUACULTURE KEY SUCCESS INDICATORS (KSIs) IN AQUACULTURE

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**Introduction:**
Success Indicators (SIs) are measurable values used to track progress toward specific objectives, while Key Success Indicators (KSIs) are a subset of these—metrics that are directly relevant and signal whether meaningful progress or failure is occurring. Objective, data-driven evaluation using scientifically validated methods is essential for credible success assessment, especially over time. This concept, originally applied in the context of public health (e.g., measuring the impact of COVID-19 vaccination programs), has also been adapted specifically to health and welfare aspects of aquaculture through the One Health framework (Stentiford et al., 2020), where indicators were developed across ecological, societal, and organismal dimensions. Building on this approach, the Cure4Aqua project aims to define and implement tailored KSIs in five key areas of fish health and welfare: vaccines and vaccination, epigenetics, disease control, diagnostics, and welfare. Each area will establish its own set of indicators through dedicated process groups, ensuring a comprehensive and systematic evaluation framework across the project's objectives.

**Materials and Methods**
The methodology for identifying KSIs within the Cure4Aqua project followed a two-phase, participatory approach.

In the first phase, an internal brainstorming session was held with members from each Work Package (WP) to generate preliminary ideas for relevant SIs and KSIs in key areas of fish health and welfare. These initial concepts served as the basis for structured discussions with external experts.

The second phase involved the formation of Process Groups (PGs) composed of selected external experts from academia, industry, public administration, and EU reference centres. Using a modified Delphi methodology, two PG rounds were conducted with an inter-round questionnaire. In the first round, experts engaged in a guided online brainstorming session to propose and discuss potential KSIs, assessing their relevance, feasibility, measurability, and data availability. Additional internally developed KSIs were also presented for feedback. Following this, a questionnaire was circulated to all experts to individually evaluate the proposed KSIs across multiple criteria (e.g., data robustness, usefulness, objectivity) and assign an overall score. The results were analysed and presented during the second PG round, where experts collectively validated and prioritized the final KSIs. This process led to the consensus-based selection of the top five KSIs to assess the effectiveness of the five key areas on aquaculture.

**Results:**
Preliminary results focusing on two of the main areas, **vaccines** and **welfare**, are presented and discussed.

Five KSIs were validated as the most relevant to assess the progression of fish vaccination in aquaculture: availability of vaccines on the market (KSI 1), number of diseases covered included in farm vaccination plans (KSI 2), decrease in disease incidence on farms (KSI 3), cost-effectiveness of vaccination strategies (KSI 4), and the number of pathogens/diseases covered per vaccine (KSI 7). Together, these indicators provide complementary insights into vaccine efficacy, effectiveness, and technological advancement in aquaculture health management.

For welfare, all proposed KSIs contribute to understanding welfare progress at different levels. However, the most highly valued indicators were the following: products certified for welfare (KSI 10), training for national competent authorities (KSI 6), and availability of welfare guidelines in a country (KSI 9). Although KS6 and KS9 are less directly connected to on-farm practices, these indicators highlight market demand, farm willingness for welfare certification, governmental interest, and regulatory engagement. A balanced mix of policy, practice, and market indicators is essential, along with clearer definitions and improved data access.

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**Conclusions:**
Preliminary results highlight key indicators for both vaccination and welfare in aquaculture. For vaccination, five KSIs were identified as most relevant, offering insights into efficacy, disease coverage, and economic impact. In terms of welfare, the most valued indicators reflect market certification, governmental commitment, and regulatory progress, pointing to the importance of aligning stakeholder efforts across the policy, production, and consumer spheres.

# ENHANCING FEED INTAKE, GROWTH, AND WELFARE IN EUROPEAN SEABASS (*Dicentrarchus labrax*) FARMING: NOVEL AQUAFEED ADDITIVES FOR A SUSTAINABLE AQUACULTURE

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## Introduction

One of the primary goals of modern aquaculture is to develop sustainable aquafeeds, largely focusing on alternative protein sources to replace traditional marine-derived ingredients. Meals derived from plant, livestock, insects and microbial sources, are the main alternatives currently employed, that however often result in lower diet's palatability. In fact, the off-flavour substances present in these ingredients often affect aquafeeds attractiveness, depressing fish feed intake, eventually having negative consequences for growth and welfare. Additionally, the resulting feed wastage derived from uneaten feed has side effects on both the farm's economy and the environment. In fact, not ingested feed remains in wastewater outflows and thus can contribute to the eutrophication of aquatic ecosystems, representing a direct financial loss as well.

To improve aquafeed palatability, marine-derived feed attractants are regularly included in aquafeeds. Particularly, meals derived from shrimp, anchovy, and squid are well-recognized feed attractants, but due to their natural origin they present both unsustainability issues and highly variable attractiveness. In fact, their attractive effect depends on their harvesting location, the raw material quality and composition, the freshness, and processing methods, posing sustainability concerns and fluctuations in the product availability. The current alternative solution is represented by a limited number of molecules, such as mixture of free amino acids, nucleosides, and nucleotides, or substances such as betaine and taurine, that can be used as feed attractants. Particularly, among these substances, amino acids have been shown the strong attractive effects, although the limitations and disadvantages of their use as feed attractants are well-known. In this context, synthetic flavours are emerging as a promising and sustainable alternative to conventional feed attractants, obtained through standardized processes. Preliminary studies conducted throughout the zebrafish life cycle (*Danio rerio*) demonstrated that the dietary supplementation of attractive synthetic flavours had positive effects on appetite stimulus, feed ingestion, reward pathways and, consequently, on the growth of fish. Zebrafish is widely recognized as an optimal model for finfish nutrition research, and preliminary findings in this species can serve as a foundation for further studies in farmed fish.

In this regard, the aim of the present study was to test the effectiveness same synthetic flavours previously tested in zebrafish, on European seabass (*Dicentrarchus labrax*), during a 90-day feeding trial, evaluating the fish physiological responses. The European seabass is a strictly carnivorous species with a high market value and of particular importance in the aquaculture sector of the Mediterranean region. The application of synthetic flavours as feed attractants can help reduce feed wastage and improve farm's productivity. This approach also supports the shift toward more sustainable and eco-friendly practices in aquaculture.

## Materials and Methods

*Experimental diets.* Two attractive (F1, cheese; F2, caramel) and one repulsive (F3, coconut) flavours, previously tested throughout zebrafish life cycle, were produced by To Be Pharma S.r.l. (S. Egidio alla Vibrata, Teramo, Italy). The selected flavours were then included in a commercial diet for European seabass (Ecoprime, Veronesi, VR, Italy), rich in land animal proteins (LAPs). Then, four experimental diets were prepared starting from the commercial CTRL diet by adding Propylene Glycol (basic solvent used for flavours' production), F1, F2, and F3 flavours (named PG, F1, F2, or F3 diets, respectively). The PG and all the flavours were added at 1% (w/w) to the CTRL diet.

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*Experimental design.* Five hundred and forty European seabass juveniles (initial body weight: 72.48 ± 8.04 g) were initially acclimated for two weeks. At the end of acclimation period, fish were randomly divided into six experimental groups (in triplicate) as follows: (i) CTRL group, fish fed the CTRL diet; (ii) PG group, fish fed PG diet; (iii) F1 group, fish fed F1 diet; (iv) F2 group, fish fed F2 diet; (v) F3 group, fish fed F3 diet; (vi) ROT group, fish fed the two attractive diets (F1 and F2), each administered singularly in a weekly rotation scheme, to avoid a possible olfactory receptors adaptation in response to a long-term stimulation. The duration of the feeding trial was 90 days during which fish were hand-fed the experimental diets at 1.5% of body weight, daily monitoring the feed consumption. At the end of the feeding trial, all the fish were euthanized and then individually weighted.

*Analyses.* Biometric data were used to calculate the zootechnical parameters, and the daily feed intake was evaluated. In addition, samples from brain, liver, and distal intestine, were collected for: i) histological analyses in liver to determine the hepatic lipid and glycogen deposition and in distal intestine to verify eventual alterations or signs of inflammation; ii) molecular analyses to evaluate the gene expression of markers involved in fish brain reward system (*drd3*), serotonergic system (*5ht1a*), and immune response (*il1b*, *il10*, *tnfa*).

**Results and Discussion**

The PG and all the tested synthetic flavours had no adverse effects on liver and distal intestine, as widely demonstrated by the histological analyses. However, fish fed caramel and coconut diets showed an upregulation of immune-related markers in distal intestine, despite the clear absence of inflammatory process evidenced by the histological analyses. However, the various roles of interleukins may explain result inconsistencies, highlighting the need for more research on how synthetic flavours affect cytokine gene expression. Despite this, fish fed F2 (caramel) diet showed a higher feed intake, confirming, even in this species, the attractive effectiveness of the caramel flavour, as already demonstrated in zebrafish. This led to direct consequences on the amount of energy available for fish to support growth performance and storage of nutrients. In fact, caramel group showed the highest weight gain and growth rates and exhibited a significantly higher fat percentage in the liver. Finally, the positive modulation of monoaminergic pathways in F2 group may contribute to the hedonic value of the diet and the desire to consume feed, driven by reward sensation.

Overall, the caramel flavour notably improved feed palatability, enhancing its intake and fish growth, highlighting the role of sensory cues in feeding motivation. This study confirmed the potential of synthetic flavours to enhance aquaculture practices in a sustainable and cost-effective approach for the sector.

# CIRCULAR AQUACULTURE: WASTE VALORIZATION FROM INSECT REARING FOR SUSTAINABLE AQUAFEED AND AGRICULTURAL APPLICATIONS (ACInA PROJECT)

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**Introduction**

Aquaculture is a critical contributor to the global economy, with an estimated production of 88 million tons of aquatic animals in 2020. As the fastest-growing sector within animal production, it holds significant potential for expansion in the coming decades. However, the development of innovative and sustainable aquafeed formulations remains a crucial factor in supporting its continued growth and long-term viability.

Chitin, the second most abundant polysaccharide in nature after cellulose, has emerged as a promising additive in aquafeeds, with potential to improve growth, intestinal health, and immune response of aquatic organisms. Traditionally, meals derived from shrimp and yeast have been the main sources of chitin in aquaculture feeds. However, the increasing interest in insect meal as a component of aquafeeds has positioned insects as a promising alternative protein source. Notably, insect farming generates biological by-products such as pupal exuviae and the exoskeletons of deceased adults, which are rich in chitin. Developing alternative processing strategies for these waste materials could enable the production of value-added products, potentially serving as nutritional supplements in the diets of various fish species. Rainbow trout (*Oncorhynchus mykiss*) is the most extensively cultivated freshwater species in both Europe and Italy, ranking highest in terms of production volume and economic significance. Despite its prominence, efforts to improve the sustainability of rainbow trout aquaculture remain a central focus of current research. In addition to the ongoing need for optimizing feed formulations, it is well-recognized that aquaculture operations generate considerable amounts of organic waste, including uneaten feed, fish faeces, and processing by-products. Effective waste management is therefore essential to reducing environmental impacts and improving resource efficiency.

In this context, innovative strategies such as waste valorization and the implementation of circular economy models are being investigated to mitigate pollution, enhance the recovery of valuable resources, and produce value-added products from aquaculture residues.

Within this context, the ACInA project adopts circular economy and zero-waste principles by linking aquaculture, insect farming, and agriculture to develop and validate waste valorization processes. This integrated strategy aims to improve sustainability, reduce environmental impact, and enhance resource efficiency across sectors.

Insects will be reared on substrates made from agri-food by-products, enabling the sustainable conversion of waste into high-value biomass. Insect-derived by-products will be used to formulate functional aquafeeds, while aquaculture waste (e.g., fish viscera and faeces) will be repurposed into compost and fertilizers for agricultural use.

Specifically, chitin extracted from insects will be incorporated into rainbow trout diets to evaluate its effects on fish growth and welfare.

**Materials and Methods**

In detail, the project focuses on: i) collecting exuviae and deceased adult specimens of the Black Soldier Fly (*Hermetia illucens*) reared on agricultural waste; ii) extracting chitin – alone or with macronutrients from both larvae and adult specimens – to formulate functional feeds for rainbow trout (*Oncorhynchus mykiss*); iii) revalorizing aquaculture waste into organic-based fertilizers for soil conditioning in agriculture, supporting the complete closure of the production cycle.

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*Exuviae collection.* Initially, agricultural organic by-products will be used as rearing substrate for *Hermetia illucens*, within a vertical farming system. During the larval stage, insects will be kept at environmental parameters suitable for their growth: temperature at 27-28 °C, humidity 70% and no light. At the end of insects' reproduction cycle, larvae, exuviae alone, and deceased adults will be collected, dried, and grinded through a mill. The recovered products will then be used for the preparation of trout experimental diets.

*Experimental diets.* Starting from a commercial diet specific for rainbow trout, which will be used as Control diet (C –), four experimental diets will be obtained: (i) C+ diet, (control diet + 5% w/w of larvae insect meal); (ii) E diet, (control diet + 1% w/w insect exuviae); (iii) A diet, (control diet + 1% w/w adult insect meal).

*Experimental design.* Three hundred rainbow trout (initial body weight: 100-120g), will be initially acclimatized for a minimum of two weeks at the experimental fish center (Fondazione Edmund Mach; Centro di Trasferimento Tecnologico, San Michele a./A., Italy). After this period, fish will be divided into 12 open circulating tanks (800L; three tanks per experimental group), according to the four experimental diets, obtaining four experimental groups. Fish will be hand-fed the experimental diets 5 days per week from 2% to 1.5% of the biomass adjusting it by mean body weight. At the end of the feeding trial, (upon reaching commercial size, 500g, 16 weeks) 5 fish per tank (15 per experimental group) will be weighted and zootechnical parameters will be measured.

*Analyses.Biometric and zootechnical indexes:* (i) growth performances calculating the specific growth rate (SGR%), and weight gain (WG); (ii) feed conversion ratio (FCR), to evaluate feed efficiency; *histological analyses* for hepatic lipid and glycogen deposition, and in distal intestine, histopathological indexes; *Molecular biology* expression of genes involved in immune response (*il1b*, *il10*, *tnfa*) and on chitin digestion (*chia*) assessed in distal intestine and stomach, respectively.

Finally, waste matter from reared fish (mainly faeces and uneaten feed) will be collected to be used for the other tasks of the project (Department of Science and Engineering of Matter, Environment and Urban Planning – SIMAU). Aquaculture wastes will be characterized in terms of carbon and nutrient (nitrogen and phosphorus) content will be subjected to co-composting processes to obtain high-quality fertilizer.

**Results and Discussion**

ACInA project adopts circular economy and zero-waste principles by linking aquaculture, insect farming, and agriculture to develop and validate processes for waste valorization. This integrated strategy aims to improve sustainability, reduce environmental impact, and enhance resource efficiency across sectors.

In this context chitin, a biopolymer derived from insect exoskeletons reared on vegetable by-products, was investigated for its potential benefits when included in rainbow trout diets. Its incorporation may enhance gut health, boost immune response, and improve overall growth performance. To better understand the role of this biopolymer laboratory analyses have been adopted and integrated. Simultaneously fish waste will be subjected to co-composting processes to obtain high-quality fertilizers to be used in agriculture. This will allow the project to be completely circular.

The approach will include interdisciplinary aspects, integrating the research areas of Chemical, Environmental and Sanitary Engineering and Biology. Integration and validation of mass and energy balances will be conducted to evaluate the efficiency, sustainability, and scalability of the proposed technological solutions through Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) analyses. Finally, dissemination of all the results will include reports, scientific and informative articles, and participation in national and international events.

ACInA project (Bando Ricerca e Sviluppo 2023/24 Fondazione Caritro project N° 2023.0598) is supported by Fondazione Cassa di Risparmio di Trento e Rovereto.

# BENCHMARKING SUSTAINABILITY IN AQUACULTURE: INSIGHTS FROM IRELAND's INAUGURAL SECTORAL REPORT

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**Introduction**
In response to growing global seafood demand and the imperative for sustainable food systems, Bord Iascaigh Mhara (BIM), Ireland's Seafood Development Agency, has published its first sustainability report on the Irish aquaculture sector. Sustainability reporting in aquaculture is essential for tracking progress, identifying areas for improvement, and informing policy and investment decisions. Sustainability reports provide transparent, science-based data that enable industry, stakeholders, regulators, and the public to assess environmental, economic, and social performance—ensuring that growth in the sector aligns with national and EU sustainability objectives (EC, 2019, 2020, 2021, DAFM 2023). This report establishes a baseline assessment of the sustainability performance of the Irish salmon, rope mussel, and oyster farming sectors, using data from 2017 to 2019.

The initiative aims to supports both the European Union's Strategic Guidelines for the Sustainable Development of EU Aquaculture (2021–2030) (EC, 2021) and Ireland's National Strategic Plan for Sustainable Aquaculture Development (DAFM, 2023).

**Materials and Methods**
The evaluation framework spanned four key pillars: environmental impact, economic performance, social contribution, and innovation. Data collected as part of national annual surveys and other national reports provided background information which allowed for analysis and investigation across the four pillars. In total 24 impact indicators were used as part of the analysis Environmental indicators assessed greenhouse gas emissions, feed resource efficiency, edible protein energy return on investment, meals produced and spatial use. Economic indicators included Gross Value Added (GVA), running cost to turnover ratio, labour productivity, and net profit. Social impacts were assessed through employment multipliers, workforce demographics, and gender diversity. Innovation was measured by the adoption of new products, processes, and organisational forms.

**Results**
Environmental analyses demonstrated that Irish aquaculture operates as a low-carbon, resource-efficient sector, producing over 154 million meals annually. Edible protein energy return on investment also demonstrated strong results, indicating efficient returns. Year-to-year variability in environmental performance was noted but overall sustainability indicators remain strong. Economically, while GVA decreased in certain sectors, sales value per unit area improved. Socially, the sector generated significant employment and wage multipliers; however, challenges remain with an ageing workforce, low recruitment rates, and limited female participation. A high level of innovation was observed, including advancements in process control and organizational structures.

**Discussion**
This inaugural sustainability report provides vital, transparent data that strengthens Ireland's ability to meet EU sustainability targets and national aquaculture goals. It aligns with the EU's strategic objectives to foster competitive and resilient aquaculture, while supporting Ireland's commitment to building a climate-neutral and socially inclusive seafood sector. The report establishes a critical foundation for evidence-based policymaking, strategic planning, and continuous sectoral improvement. The report also provides data which can be used in the curation and further development of the social license for these aquaculture activities in coastal and rural communities.

**Conclusion**
The findings affirm that the Irish aquaculture sector is well-positioned to contribute to EU and national sustainability ambitions. Ongoing monitoring, innovation, and adaptive management are essential to maintaining progress and securing a sustainable future for aquaculture in Ireland and beyond.

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# SUPPORTING SUSTAINABLE AQUACULTURE THROUGH INDEPENDENT SCIENTIFIC ADVICE

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In the North Atlantic, wild capture fisheries are longstanding and deeply rooted activities, governed by well-established regulatory frameworks. These are shaped by global agreements and implemented through relatively cohesive, quantitative management systems across the region. The sector benefits from clear governance structures, coordinated data streams, robust scientific cooperation, and well-organized stakeholder networks—factors that together underpin a mature and effective science-policy interface essential for sustainable fisheries management.

In contrast, aquaculture in the region, while growing in importance, is characterized by more fragmented governance. As a national competency, approaches to aquaculture development and regulation vary widely, and the systematic exchange of best practices is limited. The EU has taken important steps to support the sustainable growth of the sector, notably through the *Strategic Guidelines for a more sustainable and competitive EU aquaculture*[1]. These guidelines urge Member States to define and monitor environmental performance using appropriate indicators, while promoting the alignment of aquaculture with EU environmental, social, and economic objectives.

However, key questions remain: What constitutes a "best practice"? How much monitoring is sufficient? What do the data mean? And who determines what qualifies as "good" environmental performance?

As the demand for responsibly produced aquatic foods continues to rise, aquaculture must meet complex and often competing sustainability goals. Addressing these challenges requires more than localized decision-making—it demands coordinated, science-based approaches grounded in transparency, accountability, and collective learning.

The International Council for the Exploration of the Sea (ICES) has a long-standing role in providing independent, consensus-based scientific advice to national governments, regional fisheries bodies, and the European Union—primarily in the context of wild capture fisheries. Today, ICES is also advancing work on aquaculture as part of its broader commitment to the Ecosystem Approach. This includes enhancing understanding of marine ecosystems and the services they provide and using this knowledge to develop advice that supports conservation, management, and sustainability across sectors.

This presentation will showcase two successful case studies where independent scientific advice contributed to management outcomes in the Northeast Atlantic—one in commercial fisheries and one in Atlantic salmon aquaculture. It will conclude with a discussion on how similar science-policy collaboration could support sustainable marine aquaculture in EU Member States, particularly through the identification and application of relevant environmental, social, and economic indicators through the sharing of best practices and independent scientific advice. Such efforts can improve the tractability, transparency, and effectiveness of aquaculture governance and help translate policy ambitions into measurable outcomes.

1. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=COM:2021:236:FIN

# A NEW STRATEGY TO PREVENT EMERGING *Lactococcus garvieae* INFECTIONS BY USING AURAAQUA, A MIXTURE OF NATURAL PLANT EXTRACTS AND ORGANIC ACIDS, IN VITRO AND EX VIVO

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## Introduction

*Lactococcus garvieae* (*L. garvieae*), a Gram-positive lactic acid bacterium belonging to the Streptococcaceae family, has gained notoriety as a significant pathogen in aquaculture, particularly in freshwater and marine fish, including high-value species like rainbow trout and European Sea Bass —causing piscine lactococcosis. The disease is marked by high morbidity, haemorrhagic symptoms, and systemic infections. Its adaptability and capacity for biofilm formation complicate eradication and contribute to persistent outbreaks. Conventional antibiotic treatments are losing effectiveness due to rising antimicrobial resistance, necessitating the exploration of safer, more sustainable alternatives. Plant extracts and organic acids (OAs) have emerged as promising candidates due to their natural antimicrobial, anti-inflammatory, and immunomodulatory effects. This study evaluates the efficacy of a plant extract and organic acid mixture AuraAqua (Aq), against *L. garvieae* in vitro (CHSE-214 fish epithelial cell line) and ex vivo (fish red blood cells), focusing on its impact on bacterial growth, virulence gene expression, host immune responses, and cytotoxicity.

## Materials and Methods

This study employed CHSE-214 epithelial cells and rainbow trout red blood cells to model infection. The MIC and MBC of Aq were determined using standard dilution techniques. The anti-virulence activity of Aq at the established MIC (0.125%) was assessed by, measuring bacterial growth, EPS gene expression, biofilm formation, cytotoxicity via LDH release, adhesion of *L. garvieae* to epithelial cells and gene expression of haemolysins (hly1, hly2, hly3). Immunomodulatory and inti-inflammatory effect of Aq was assessed by measuring host inflammatory cytokine responses (IL-1β and IL-8). Finally, the protective effect of Aq was measured by challenging trout red blood cells with *L. garvieae* and measuring haemolysis in an ex vivo assay. Gene expression was analysed using quantitative RT-PCR, and statistical analysis was performed using t-tests and ANOVA.

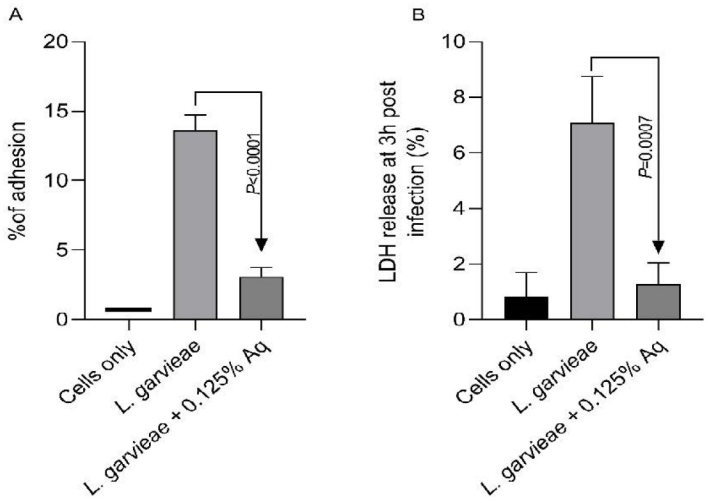


Fig. 1. The impact of Aq on the adhesion of L. garvieae to CHSE-214 cells (A) and on bacteria induced cytotoxicity (B). The p value indicates a very significant difference when 0.125% Aq was added to the infection assay ($p < 0.0001$).

*(Continued on next page)*

**Results**

Aq showed inhibitory and bactericidal activity at 0.125% (MIC) and 0.75% (MBC), respectively.

At a concentration of 0.125% Aq significantly reduced number of *L. garvieae* vilulence factors including growth, EPS gene expression (epsA, epsB, epsC, etc.), and biofilm formation. In addition, Aq inhibited bacterial adhesion to CHSE-214 cells and reduced cytotoxic effects (Fig. 1).

In ex vivo experiments a significant reduction in haemolysis was observed when Aq was co-administered with *L. garvieae* in red blood cell assays ($p = 0.02$). Expression of *L. garviae* virulence genes hly1 and hly3 was significantly downregulated, while hly2 showed no significant change. IL-1β and IL-8 expression was significantly reduced in infected CHSE-214 cells treated with Aq, highlighting its anti-inflammatory potential.

These effects collectively indicate Aq's ability to suppress bacterial virulence and mitigate host inflammatory responses without harming host cells.

**Conclusion**

This study demonstrates that a natural blend of plant extracts and organic acids (Aq) can significantly reduce the pathogenic potential of *L. garvieae* by inhibiting key virulence factors such as exopolysaccharide production, haemolysin gene expression, and host cell adhesion. Additionally, Aq reduces inflammation and cytotoxicity in host cells, suggesting its dual benefit as both an antimicrobial and immunomodulator. These findings position Aq as viable non-antibiotic alternatives for controlling *L. garvieae* infections in aquaculture, contributing to sustainable fish health management strategies and addressing the growing need for antibiotic-free disease control.

# THE EFFECTS OF CRYOPRESERVED SPERM USE ON OFFSPRING TOLERANCE TO ENVIRONMENTAL STRESSORS IN SALMON RESTOCKING PROGRAM

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## Introduction

The Atlantic salmon is an emblematic species of Western Europe rivers that flow into the North Atlantic Ocean, yet its populations have been in decline throughout the 20th century. Several European programs in Western Europe (*ie*, such as those in Denmark, Germany, Wales or Belgium), aim to sustainably restore salmon habitats and populations by implementing restocking actions-including the release of emerging yolk fry and parr, removal of migration barriers, and habitat restoration measures. Nevertheless, adult salmon returns remain relatively low and show considerable interannual variability. The use of cryopreserved sperm from genetically valuable males in aquaculture production provides a promising tool for conservation. It allows for long-term storage of genetic material and helps maintaining genetic diversity (Magnotti et al. 2018). This may, for example, help compensate for the lack of returning anadromous male in certain years. Preliminary experiment using cryopreserved semen resulted in hatching of 60% of fertilized eggs suggesting that such cryopreserved sperm could be used for annual breeding programs when the stock of anadromous males is too low. However, cryopreservation is also known to cause cell damage, affecting the plasma membrane, mitochondrial membrane potential, and sperm motility, ultimately impacting reproductive success (Erraud et al. 2024). Although no significant effects of cryopreservation was observed on fertilization rates, growth, survival nor pathogen resistance during the embryo stage, Nusbaumer et al. (2019) found that fertilization by cryopreserved sperm significantly reduced larval growth after hatching.

In addition, salmon released through restocking programs will be increasingly exposed to the impacts of climate change. It is now well established that rivers are experiencing accelerated warming during the summer months, along with a higher frequency of extreme temperature events. These changes are particularly concerning for cold-water species such as Atlantic salmon, which are highly sensitive to elevated water temperatures (Buisson et al., 2008).

Our experiment aimed at evaluating the effect of using cryopreserved sperm from the Meuse River strain (Belgian) on fry development and their resistance to environmental stressors such as river warming and bacterial infection. The effects of two stressors experienced by hatchery-reared juveniles upon release were tested: daily thermal fluctuations specific on the recipient river and an infection with a bacterial pathogen. The environmentally realistic thermal conditions tested in this study were set based on temperature records from three Belgian rivers (Ry de Ferron (constant 10°C), Berwinne (6.5–12.3°C), and Ry d'Oxhe (4.5–16°C) rivers).

## Material and Methods

Artificial reproductions were performed using anadromous male breeders (using both fresh and cryopreserved sperm) and 2+ female breeders. Eggs were collected, pooled and divided into batches of 300 eggs. They were then fertilized with fresh and cryopreserved sperm to obtain a standard sperm-to-egg ratio of 10°C and transferred into individual vertical micro-incubators supplied with recirculated freshwater at 9.0 ± 0.4°C throughout the incubation period. Fifteen days post-hatching, salmon fry (N = 360) from fertilization with fresh and cryopreserved sperm were subjected to thermal stress as such: a constant temperature of 9 °C, a daily fluctuation between 7-12°C, or a fluctuation between 3-15°C. Biometric variables such as size, weight, and yolk sac size were measured at the start of the experiment, after 24 h and 11 days of thermal stress exposure. Nine individuals per condition were euthanized using a final concentration of 200 mg $L^{-1}$ buffered MS-222 to collect biological material for molecular analyses via qPCR.

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Following thermal stress, half of the remaining organisms were exposed through a bath infection to the bacterial pathogen *Aeromonas salmonicida achromogenes* at $1.41 \times 10^9$ CFU/mL for 6 hours at 9°C; the other half was kept as a control group uninfected. After 6 h of bacterial challenge, half of the exposed fry and half of the control fry were euthanized and sampled for molecular analyses using qPCR analyses.

**Results and discussion**

This study represents the first investigation assessing the impact of sperm cryopreservation on the development of yolk-sac fry intended for restocking, as well as their resistance to two successive environmental stressors: thermal stress and bacterial challenge. Particularly, the higher daily thermal fluctuations (3–15°C) resulted in reduced growth rates and yolk reserves. These phenotypic observations were sustained by transcriptomic analyses, which revealed that progeny derived from cryopreserved sperm exhibited upregulated expression of genes associated with antioxidant defences and energy metabolism. These high fluctuated temperatures also induced an increase in intracellular reactive oxygen species (ROS), leading to heightened energy expenditure to counteract oxidative stress. Consequently, energy allocated to stress responses was unavailable for somatic growth. It is therefore likely that fry originating from cryopreserved sperm possess lower critical thermal minima and maxima compared to those produced with fresh sperm.

Moreover, the bacterial challenge demonstrated that fry from the cryopreserved group were able to face the bacterial infection with an effective immune response only when they had not been previously exposed to thermal stress (i.e., 3-15°C). Our results suggest that sperm cryopreservation could have reduced the capacity of progeny to cope with multiple environmental stressors. While the use of cryopreserved sperm remains a viable strategy for restocking salmon populations, caution is warranted when releasing these individuals into rivers subject to substantial daily temperature fluctuations, such as the Ry d'Oxhe in Belgium.

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# NUTRIENT-DRIVEN GROWTH RESPONSES OF HAWAIʻI NATIVE LIMU MANAUEA LOLOA *(Gracilaria parvispora)* AND INVASIVE *Gracilaria salicornia*: CENTERING NATIVE HAWAIIAN AQUACULTURE RESTORATION THROUGH PURPOSE, PLACE, AND PARTNERSHIP

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Limu (native macroalgae) plays a critical role in Hawaiʻi's nearshore ecosystems, acting as a natural biofilter, providing habitat and sustenance for marine life, and serving as a valuable resource for human consumption while holding deep cultural significance. However, the decline of native limu species due to environmental threats and human impacts has underscored the need for ongoing restoration efforts. Furthermore, little is documented about the specific conditions native limu require to thrive in cultivation systems that support broader restoration efforts. This study aims to determine the optimal growth conditions for Limu Manauea Loloa (*Gracilaria parvispora*) to support restoration and sustainable aquaculture.

In addition, the study includes a Hawaiʻi invasive species, *Gracilaria salicornia,* to compare growth responses across a nutrient availability gradient, helping to understand competitive dynamics and environmental thresholds influencing both species. The study evaluates key environmental factors influencing limu growth by replicating salinity and nutrient levels found at sites with varying cultivation success, such as loko iʻa and restorative aquaculture facilities. It assesses how these conditions affect biomass production and photosynthetic efficiency in Limu Manauea Loloa and *G. salicornia*. Findings will inform restoration strategies aligned with ecological and cultural priorities, fostering sustainable limu stewardship.

Loko iʻa, traditional Hawaiian fishponds, represent a foundational form of Indigenous aquaculture and coastal resource management. Once abundant across the islands, many loko iʻa have deteriorated due to neglect, development, and invasive species. Restoring these systems not only revitalizes traditional food production and strengthens biocultural connections but also creates ideal habitats for native limu to flourish. By understanding the conditions that support limu growth in loko iʻa, this research supports ongoing fishpond restoration efforts and highlights the interconnectedness of limu health and ecosystem stewardship.

This research is a collaborative effort rooted in community-based resource management, guided by the knowledge of local practitioners and organizations. By bridging cross-disciplinary efforts, we aim to develop practical recommendations that empower community-led restoration. Through purpose, place, and partnerships, this study contributes to revitalizing Hawaiʻi's native marine resources.

Data collection is expected to conclude in early summer 2025, with results to follow shortly thereafter.

# PITUITARY mRNA EXPRESSION IN GREATER AMBERJACK *Seriola dumerili* EXHIBITING IMPAIRED SPERMATOGENESIS

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## Introduction

The greater amberjack *Seriola dumerili* is a promising aquaculture candidate for the Mediterranean Sea. However, hatchery-produced individuals exhibited severe reproductive dysfunctions, characterized by (a) incomplete gametogenesis and (b) the absence of spontaneous spawning, when reared in tanks or sea cages, respectively (Lancerotto et al., 2025). We present here a comparative analysis of the pituitary transcriptome of wild and hatchery-produced greater amberjack males sampled during the active gametogenesis, as part of a research program aimed at describing the effects of rearing in captivity on gene expression along the reproductive axis of this species.

## Material and Methods

Pituitaries from wild and hatchery-produced males were sampled in 2022 on board a fishing vessel and in a fish farm in Salamina (Greece), respectively, and preserved in RNA later®. To assess the reproductive state, testis samples were fixed in Bouin's solution, embedded in paraffin wax, sectioned and stained with haematoxylin-eosin. Fish were then assigned to one of the following groups: WILD (wild fish undergoing active spermatogenesis; N = 4); NormalF (farmed fish undergoing active spermatogenesis; N = 4); DysF (farmed fish with arrested, dysfunctional, spermatogenesis; N = 2). Pituitary total RNA extraction was performed by RNeasy® Plus Mini kit (Qiagen, Germany), mRNA libraries were prepared by SureSelect Strand Specific RNA Library Preparation kit (Agilent Technologies, California, U.S.) and paired-end sequencing was performed on the Illumina NextSeq platform (Illumina Inc., California, U.S.). Differential gene expression analysis was carried out using DESeq2. DAVID (https://david.ncifcrf.gov/tools.jsp) was used to perform the functional annotation of Differently Expressed Genes (DEGs) and the Gene Ontology enrichment analysis. A protein-protein interaction (PPi) network based on DEGs associated with each comparison was built using STRING (https://string-db.org). DEGs were mapped to KEGG pathways using KEGG Mapper – Search (https://www.genome.jp/kegg/mapper/search.html).

## Results and Discussion

The transcriptome analysis identified 18176 genes, of which 16234 were common to the three groups, and 889, 90, and 8 genes were specifically expressed in WILD, NormalF and DysF, respectively (Fig. 1). Differentially expressed gene (DEG) analysis revealed 301 and 376 DEGs in DysF vs. WILD and NormalF vs. WILD comparisons, respectively, while no DEGs were detected between DysF and NormalF groups (Table 1). Pathway enrichment analysis highlighted significant alterations in *purine* and *nucleotide metabolism* pathways in DysF vs. WILD, and in *antiviral defense* biological process in NormalF vs. WILD. PPi analysis identified broad regulatory networks involving 246 proteins across eleven functional categories in DysF vs. WILD, and 382 proteins across ten categories in NormalF vs. WILD. KEGG analysis showed DEGs enriched in 62 (DysF vs. WILD) and 84 (NormalF vs. WILD) pathways. Notably, several dysregulated pathways were common to both farmed fish groups and were associated with reproductive functions, including GnRH and ErbB signaling pathways. Among DEGs, genes encoding for Neuron-derived neurotrophic factor, Proenkephalin, Gamma-aminobutyric acid, Insulin-like Growth Factor 1, Beta-arrestin, Muscarinic acetylcholine and Cholinergic receptors were found.

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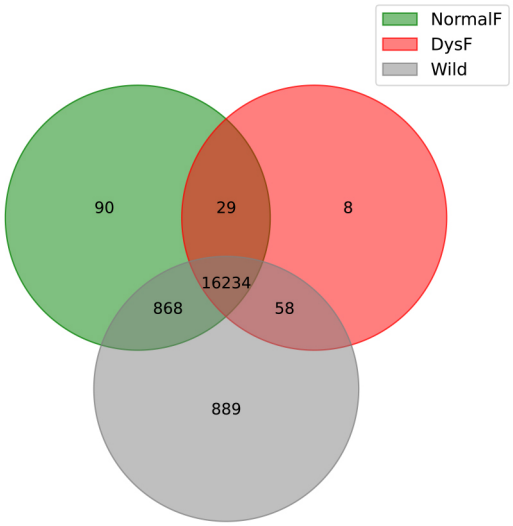


**Fig. 1.** VENN diagram of shared and unique expressed genes of wild (WILD), dysfunctional farmed (DysF) and non-dysfunctional farmed (NormalF) fish.

**Table 1.** Number of upregulated and downregulated genes in the three groups of greater amberjack males.

| | TOTAL GENES | DEGs | UP | DOWN |
|---|---|---|---|---|
| **DysF vs. WILD** | 18,086 | 301 | 141 | 160 |
| **NormalF vs. WILD** | 18,168 | 376 | 163 | 213 |
| **DysF vs. NormalF** | 17,287 | - | - | - |

The data reveal widespread disruption of gene expression and associated pathways in hatchery-produced male greater amberjack. This disturbance influences a variety of biological processes and suggests that the spermatogenic impairment reported by Lavecchia et al. (2023) may stem from broad suppression of pituitary function, possibly driven by dysregulation within the opioid, endocannabinoid, and dopaminergic systems.

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# POTENTIAL IMPACT OF CLIMATE CHANGE ON THE SPREAD OF NERVOUS NECROSIS VIRUS: A CRITICAL THREAT TO MARINE ECOSYSTEMS AND GLOBAL FOOD SECURITY

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## Introduction

Viral Nervous Necrosis Virus (NNV), a member of the Nodaviridae family, is recognized as one of the most devastating viral pathogens in marine aquaculture worldwide. Its impact is especially severe in high-value species such as groupers (*Epinephelus spp*.), where it predominantly targets the central nervous system, causing vacuolation of brain and retinal tissues. This results in erratic swimming behavior, high mortality rates in larvae and juveniles, and considerable economic losses.

In parallel, the intensification of climate change has introduced new, pressing variables influencing host-pathogen-environment interactions, drastically altering marine ecosystems. In particular, the rise in sea surface temperatures, a direct consequence of climate change, is destabilizing marine environments and exacerbating disease outbreaks. These elevated temperatures are compromising fish immune defenses, making them more vulnerable to infections like NNV. The interplay between changing climate conditions and pathogen dynamics underscores the urgency of developing robust surveillance strategies for early detection and management of NNV. In this study, we assessed the effect of temperature on viral replication to better understand how climate-related stressors, particularly warming oceans, may amplify disease dynamics and impact aquaculture. Furthermore, we investigated the presence and viability of NNV in wild Mediterranean grouper specimens, shedding light on how the virus's geographic distribution and pathogenic potential are shifting in response to changing environmental conditions driven by climate change.

## Materials and Methods

To evaluate the impact of rising temperatures, viral replication was assessed at 25°C and 28°C using two different multiplicities of infection (MOI 1 and MOI 10). Cytopathic effects were monitored daily under an inverted microscope, and viral titers were evaluated. In addition, brain tissues collected from wild grouper specimens were processed for molecular and virological analyses. Total RNA was extracted, reverse-transcribed, and analyzed via real-time PCR targeting the RNA1 segment of NNV. For viral isolation, tissue homogenates were inoculated onto SSN-1 cell lines.

## Results

In vitro infection of SSN-1 cells resulted in marked cytopathic effects, such as cell rounding, vacuolation, and detachment, by day six post-infection, confirming successful viral replication and isolation. Temperature-based assay revealed that viral replication was significantly higher at 25°C than at 28°C across both MOI conditions. This suggests that elevated temperatures, although generally considered favorable for pathogen proliferation, may instead compromise the host's physiological state, rendering fish more susceptible to infection even if viral replication itself is reduced. In addition, molecular screening confirmed the presence of NNV RNA in grouper specimens. These findings emphasize the relevance of temperature as a modulating factor in host-pathogen interactions and support the need for adaptive management strategies in the face of global climate change.

# IDENTIFICATION OF REPRODUCTIVE CYCLE BIOMARKERS IN CARPET SHELL CLAM (*Ruditapes decussatus*) AND MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis)*

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**Introduction**

The reproductive cycle of bivalve molluscs plays a critical role in their physiology, population dynamics, and commercial value. In species such as the Mediterranean mussel (*Mytilus galloprovincialis*) and the carpet shell clam (*Ruditapes decussatus*), understanding the timing and progression of gonadal development is essential for optimizing broodstock management, improving hatchery efficiency, and supporting sustainable aquaculture practices[1,2]. Traditionally, the identification of reproductive stages relies on histological analysis of gonadal tissue, which, although informative, is labor-intensive, requires specialized expertise, and is not suited for high-throughput applications[3]. In this context, the development of molecular tools capable of accurately identifying reproductive stages rapidly and cost-effectively has become increasingly relevant. Molecular markers offer the potential for automation, reproducibility, and early-stage detection[4]. This study aims to identify reproductive stage-specific molecular markers in *M. galloprovincialis* and *R. decussatus*, and assess their potential for improving reproductive monitoring and sustainability in bivalve aquaculture.

**Material and methods**

To obtain individuals representing three key reproductive stages (juvenile, pre-spawning, and post-spawning), spawning was induced in adult specimens of *M. galloprovincialis* and *R. decussatus* under controlled laboratory conditions. Juvenile samples were obtained from the resulting progeny. Adults that released gametes during induction were preliminarily classified as post-spawning. At the same time, those from the same batch that did not spawn were initially assumed to be in the pre-spawning stage. This stage assignment was subsequently verified through classical histological analysis of gonadal tissue using hematoxylin-eosin staining. Representative individuals from each confirmed stage were selected for downstream molecular analyses. In the first phase of the study, we investigated the expression profiles of a set of candidate genes previously described in the literature (*vitellogenin, foxl2, vasa*-like gene*, piwi,* or *bmp2a*) as being involved in bivalve gametogenesis and gonadal maturation. Gene expression was quantified using quantitative PCR (qPCR), and results were analyzed to assess correlations with histologically defined stages. Since candidate gene expression did not provide sufficient resolution to distinguish between stages, a second phase employing an omics-based approach was implemented. This included the high-throughput identification of proteins and metabolites differentially expressed across the reproductive stages. Proteomic and metabolomic analyses were performed using mass spectrometry techniques, allowing for a broader and more unbiased search for potential biomarkers.

**Results and Discussion**

In the Mediterranean mussel, the expression levels of *vitellogenin* and *piwi* genes varied across the three analyzed stages. *Vitellogenin* expression was elevated in the post-spawning stage compared to the juvenile and pre-spawning stages (Fig. 1A). However, for *piwi* expression, the highest expression levels were found in the pre-spawning stage (Fig. 1B), although high individual variability was observed. Regarding metabolite concentrations in gonadal tissue, glucose levels showed clear differences among the developmental stages, with a marked increase in the pre-spawning stages. In contrast, lactate and cholesterol concentrations appeared unaffected by developmental stage, remaining at consistently low levels across all three groups (Fig. 2). In *R. decussatus*, gene expression patterns were more variable. *Vitellogenin* did not show a consistent trend across the different gonadal stages, and both males and females displayed high individual variability in expression levels (Fig. 3A). In contrast, *piwi* expression in males appeared to be more concentrated in histology-defined stages 2 and 3 (Fig. 3B), although no clear pattern was observed in females. Overall, *R. decussatus* exhibited high inter-individual variation in gene expression, with no consistent association between expression levels and gonadal stage for either marker. Although proteomic and metabolomic analyses have been initiated, the results are still under evaluation and are therefore not included in the present report. It is expected that this integrative approach will provide further insight and help clarify the patterns observed in the gene expression and metabolite data presented here.

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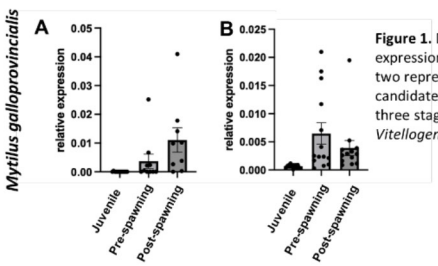


**Figure 1.** Differential gene expression in gonads of two representative candidate genes in the three stages analyzed. (A) *Vitellogenin* and (B) *piwi*.

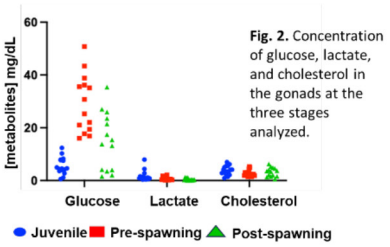


**Fig. 2.** Concentration of glucose, lactate, and cholesterol in the gonads at the three stages analyzed.

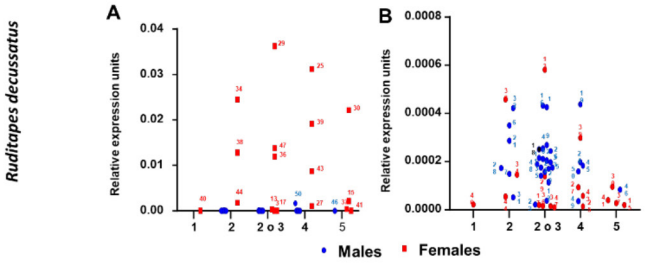


**Fig. 3**. Differential expression by sex in gonads of two representative candidates genes in the five stages previously determined by histology. (A) *Vitellogenin* and (B) *piwi*.

## Conclusions

This study reveals that transcript-level markers are unreliable for staging reproduction in bivalves due to high individual variability, especially in *R. decussatus*. However, metabolic profiling in *M. galloprovincialis* showed consistent glucose patterns linked to aerobic metabolism. These findings support proteomic and metabolomic approaches as more robust alternatives for identifying reproductive biomarkers, especially in species with variable reproductive cycles.

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# EFFECT OF THE TEMPERATURE ON THE SURVIVAL AND FEED INTAKE OF BANDED CARPET SHELL CLAM, *Polititapes rhomboides*

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## Introduction

The production of banded carpet shell clam, *Polititapes rhomboides*, has drastically declined in recent years in Galician markets, posing a serious threat to the sustainability of local shellfisheries[1]. Episodes of mass mortality have been linked to an increased prevalence of bacterial infections, with environmental conditions suggested as a contributing factor[2]. Among these, seawater temperature has emerged as a key driver. Analyses of mortality events revealed that they were often preceded by periods of above-average temperatures[3], supporting the hypothesis that thermal stress, alone or in combination with other environmental factors, may compromise the immune capacity of *P. rhomboides*[4]. Given that this species is not currently cultured and lacks a controlled seed supply, understanding its response to rising temperatures is essential for preserving the natural stocks. This study examines temperature as a key environmental factor affecting its survival and aims to support adaptive management under climate change.

## Material and methods

Specimens of *P. rhomboides* were collected from three natural shellfish beds located in the Ría de Vigo (NW Spain) (Fig.1) using a traditional artisanal harvesting technique ("*marisqueo a flote"*), which involves manually deploying a rake-like dredge ("*raño"*) from a small boat to extract clams from shallow subtidal sediments. Approximately 150 individuals were collected from each site. Upon arrival at the aquarium, the clams were held for seven days in an open-flow seawater system supplied with 100 µm-filtered seawater, allowing them to feed on naturally occurring microalgae. This acclimation period ensured stabilization before experimental manipulation. Following acclimation, clams from each site were randomly distributed among the three seawater temperature treatments: 15°C (control), 18°C, and 21°C. The experiment was conducted in three 400 L closed-circuit tanks, each maintained at one of the target temperatures. Within each tank, twelve mesh baskets were used to separate replicate groups: four replicates of ten individuals from each of the three collection sites. This setup allowed for the simultaneous exposure of all replicates to their respective temperature treatment under identical conditions. For the 18°C and 21°C treatments, seawater temperature was gradually raised over 2–3 days (~1.5°C/day) to minimize thermal stress during acclimation. Throughout the 30-day experiment, clams received a total of 5 L of a mixed microalgae suspension (T-Iso and *Rhodomonas lens*) daily. Mortality was daily recorded before the addition of the microalgae. Food intake was assessed once per week by measuring the average reduction in phytoplankton concentration in each tank, calculated as the difference between the initial and remaining algal levels after consumption.

## Results and Discussion

Across all three collection sites, clams exhibited higher mortality rates at the highest temperature treatment (21°C) (Fig. 2). Clams from the Cíes shellfish bed showed the greatest sensitivity, with mortality rates approaching 25% by the end of the experiment. In contrast, individuals from Cabo do Mar displayed intermediate mortality levels, reaching approximately 12%, while those from Tirán exhibited the lowest mortality, remaining below 5%. These results suggest that the clams' geographic origin may influence their thermal tolerance, potentially reflecting site-specific adaptation to local environmental conditions. The decrease in phytoplankton consumption by clams from week 2 to week 5 became more pronounced with increasing temperature. At 15 °C, consumption declined by only 7.49%, whereas at 18 °C the reduction reached 8.62%. The greatest decrease was observed at 21 °C, with a drop of 11.08% (Fig. 3). This trend suggests that elevated temperatures may lead to behavioral responses such as shell closure, a common protective strategy in bivalves under thermal stress. By reducing valve opening time, clams likely limit their feeding activity, which could explain the progressive decline in phytoplankton intake observed at higher temperatures.

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**Figure 1.** Location of the three shellfish beds: Cíes, Cabo do Mar, and Tirán. The figure shows a general map of Spain, followed by a detailed view of Galicia and, finally, the Ría de Vigo, where the shellfish beds are marked.

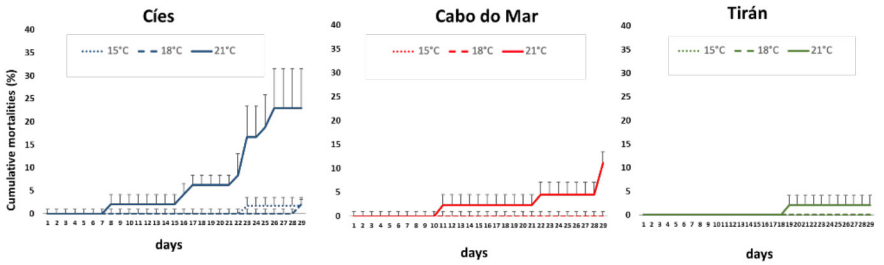


**Figure 2.** Cumulative mortality (%) of *Polititapes rhomboides* over a 30-day period under three seawater temperature treatments (15°C, 18°C, and 21°C). Data are shown separately for each collection site (Cíes, Cabo do Mar, and Tirán). Error bars represent standard error of four replicates per treatment.

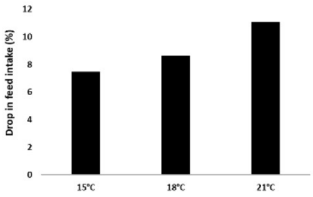


**Figure 3.** Relative change in feeding activity of *Polititapes rhomboides* between the first and last week of the experiment, calculated as the ratio of average t60/t0 values (phytoplankton concentration at 60 minutes relative to time 0) for each week, under three temperature treatments (15°C, 18°C, and 21°C). Higher values indicate a greater reduction in feeding activity over time. No error bars are shown, as the ratio represents a comparison between independent weekly averages rather than paired replicates.

## Conclusions

This study highlights that elevated seawater temperatures significantly affect the survival and feeding of *P. rhomboides*. Mortality differences among shellfish beds suggest local adaptation, while reduced phytoplankton intake under heat stress points to behavioral changes that may limit food consumption. These findings underscore the vulnerability of natural stocks to climate-induced warming and the need for site-specific management strategies to ensure their sustainability. While temperature was examined in isolation, future analyses within the framework of the RIFA project (REDEMAR. Xunta de Galicia) will consider additional environmental factors to better understand the multifactorial drivers of stress in natural populations.

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# AN INTEGRATED, LOW-COST COMMUNITY-BASED MODEL OF RESTORATIVE COASTAL AQUACULTURE WITH NUTRIENT DIVERSION AS A NATURE-BASED SOLUTION

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The global climate crisis caused by unsustainable hydrocarbon emissions has placed Humanity and our Earth into a new, sorrowful era of biosystems collapses and community catastrophes. The global scientific consensus used to urge nation states to make significant, credible emission reductions has not been enough, as wars, political malfeasance and malaise by arrogant, corrupt leaders are in ascendance. What can an individual scientist, a family, a community do if nation states are so irresponsible for everything we hold dear? Of course, we can decrease emissions and overconsumption as much as possible by moving to renewable energy, better diets, and become climate activists. These are global priorities for every citizen and family. Our community of aquatic scientists has additional scientific responsibilities to understand as much as possible and propose innovative actions to assist in the recovery of aquatic ecosystems from accelerated anthropogenic damage. Actions go beyond a need to understand IPCC projections of climate impacts from carbon emissions.

Ocean acidification is a terrifying concept; but the most urgent priority is coastal/freshwater ecosystem acidification from nutrients, especially nitrogen (N), which has been called "the forgotten element of climate change". Wastewater discharges to aquatic ecosystems from coastal urbanization and unsustainable agriculture control the Earth's coastal and freshwater N, P cycles. The Millennium Ecosystem Assessment predicted an increase in global sewage emissions of 12.0–15.5 Tg N and 2.4–3.1 TgP/year by 2050, and these may have already been exceeded. Lu et al. (2024) found that as a city grows energy use becomes more efficient and greenhouse gas emissions (GHG) decrease, but that water use increases exponentially; thus, wastewater discharges grow at a faster rate than a growing urban population. Tuholske et al. (2021) showed that 58% of coral reefs and 88% of seagrass beds of the 135,000 watersheds mapped globally were exposed to wastewater N inputs.

GHG emissions from unsustainable burning must decrease; but we are in a major nearshore, coastal/freshwater acidification crisis due to the accelerating quantities of nutrients (N to estuarine/coastal ecosystems, P to freshwaters) being discharged causing severe hyper-eutrophication and a cascade of adverse impacts to ecosystem functions and critical habitats. Coastal/freshwater acidification is due to the intensity of nutrient loading rates as there are clear links between acidification, hypoxia, intense algal blooms, and eutrophication. $pCO_2$ conditions being experienced now in coastal/freshwater ecosystems are not expected to occur in the open ocean within the next hundred years, if ever, given IPCC projections of anthropogenic $CO_2$ increases.

Nutrient discharges are the main cause of coastal/freshwater acidification, not GHG $CO_2$ dissolving into water. Many (most?) freshwater and coastal ecosystems are net heterotrophic (Duarte and Prairie 2005); they are "sewage-fuelled" marine ecosystems discharging $CO_2$ to the atmosphere. Jamaica Bay off New York City is a hypereutrophic estuary that receives ~90% of its N from four wastewater treatment plants that discharge ~ 1 billion liters of effluent daily. pCO2 concentrations in bottom waters in summer exceed 2000 μatm (the atmosphere is ~437 ppm now) (Wallace et al. 2014). Almost 30 years ago, Frankignoulle et al. (1998) found an area of European estuaries emitted ~3000 MT $CO_2$/d to the atmosphere; $CO_2$ emissions from the Scheldt estuary, Belgium/Netherlands were 2/3 due to heterotrophy and 1/3 due to ventilation. Bastviken et al. (2024) found that, given recent global freshwater $CO_2$ emissions of ~1.4 PgC ($CO_2$ eq)/year, freshwaters were a very important component of the global GHG balance comprising ~79% of the terrestrial GHG sink. They stated that, "the terrestrial GHG sink may be smaller than currently believed, and data on GHG release from inland waters are needed in future revision of net continental GHG fluxes."

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"Solutions" to accelerating urban wastewater discharges to coastal/freshwater systems are dominated by proposals by policy-makers, regulators, sanitary engineers and scientists for the massive expansion of capital-intensive sewer systems, the requiring installations of "alternative septic systems, or dilution "offshore" in the hope of restoring the water quality of valuable aquatic ecosystems. Such calls require large and unprecedented government expenditures and residential tax increases.

An alternative community-based, integrated restorative aquaculture with UD nutrient diversion model is underway in a coastal area of New England, US. It illustrates the potential of nature-based, solutions which can accelerate the development of low trophic level aquaculture and help ameliorate the nutrient/carbon crisis of aquatic ecosystems. Efforts combined applied science from Europe and North America in shellfish aquaculture with urine diversion (UD). UD is the concept of separating and collecting urine from wastewater at the source for reuse as fertilizers (struvite). Approximately 80% of the N and ~60% of P in wastewaters are from urine; but urine is only ~1% of wastewater volume (Wald 2022). The Green Center developed low-cost UD and the community approved zoning and guidelines for shellfish aquaculture as public-private partnerships. The community provided shellfish farmers with floating gear and seed. Farmers paid a $20,000 access fee to the community and were obligated to grow a preset minimum biomass on all leases. In 2023, shellfish farmers harvested 1.43 million oysters from 0.6 ha. The community used a peer-reviewed science finding that each oyster harvested removed ~0.28 gN. Thus, in 2023, a total of ~400 kgN was removed from ~0.6 ha of oysters. N removal by oysters offset ~$4.5 million in costs for new sewer infrastructure and harvests produced ~$350,000 in revenue.

Restorative shellfish aquaculture removes nutrients *in situ* already present, while UD prevents new nutrients from entering aquatic systems. Citing 2023 data scientists calculated that some N-impaired estuaries from septic leachates would not require expensive sewering or alternative septic systems at all. Although UD alone could meet nutrient reduction goals, restorative shellfish aquaculture improved water quality and would help reduce capital investments. Restorative shellfish aquaculture and UD would be much more rapidly implemented and would improve aquatic ecosystems sooner than the decades-to-implement sewers and septic systems.

Alternative, integrated ecological approaches to aquatic ecosystem restoration are nature-based solutions that create new community revenues, jobs, produce nutritious, tasty foods, and recover valuable fertilizers. They use fewer natural resources, are more environmentally beneficial and sustainable than conventional solutions, less expensive, and would avoid future, very large GHG emissions. A nature-based solution incorporating shellfish aquaculture and UD demonstrates how homeowners and coastal communities can work together to better accomplish environmental restoration and provide an alternative to investments in capital intensive sewer/septic systems being promoted worldwide as the sole solution to our aquatic/coastal crisis.

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# DIETARY INCLUSION of *Euglena gracilis* BIOACTIVE COMPOUNDS CAN MODULATE THE EUROPEAN SEABASS IMMUNE RESPONSES FOLLOWING TWO DIFFERENT INFECTION MODELS

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**Introduction**

The host nutritional status can be included among the significant aspects that influence immune responses, modulating the resistance to infection (Kiron, 2012). Nutrition can have substantial health implications for animals. Particularly in fish, best practices on diet formulation are of significant importance, as feeds probably represent the most lavish operational expenditure to the aquaculture industry (Trichet, 2010). Nutritional intervention can be a critical component of disease prevention or an essential aspect of disease management, and microalgae can be vital functional ingredients. The term "functional feed" describes fish feeds that have added benefits beyond the fish essential nutritional requirements. As the health status and growth are expected to be improved by consuming these functional feeds, a shift away from chemotherapeutic and antibiotic treatments would be possible. While many additives or feed ingredients derived from microalgae are available for inclusion in functional feeds, little attention has been paid to the role of *Euglena gracilis* as potential immunomodulator during fish early live stages. In fact, *E. gracilis* produces high levels of β-1,3-glucan, omega-3 fatty acids, protein, and vitamins C and E. The present study aimed to assess the immunomodulatory potential of dietary *E. gracilis* inclusion in diets for European seabass (*Dicentrarchus labrax*).

**Methodology**

European seabass juveniles (initial average weight 10.7±1.2 g) were fed five isoproteic and isolipidic experimental diets for two weeks. A-H and A-L: *E. gracilis* (strain A) broken cells at high or low concentrations, respectively; and B-H and B-L: *E. gracilis* (strain B) extract in combination with a carbohydrate-rich residue at high or low concentrations, respectively. At the end of the feeding trial, seabass from all dietary treatments were submitted to two different bacterial challenges by either inoculating *Photobacterium damselae piscicida* (*Phdp*) into the peritoneal cavity, or bath challenged with *Tenacibaculum maritimum* for 2 hours in aerated seawater. Following infection, blood and tissues (head-kidney, liver, gut, and skin) were collected after 24 hours for assessing haematological and immune parameters, oxidative stress, and gene expression. Cumulative mortality was followed for 12 days.

**Results**

Results from the present study showed a similar host response to both infection models regardless dietary treatments. Indeed, leukopenia was observed in all infected fish as a result of white blood cells migration to the sites of pathogen inoculation. In contrast, haematological parameters such as haematocrit and red blood cell counts increased at 24 hours due to host increased oxygen demands during the inflammatory response. Although no significant changes in disease resistance were observed due to dietary treatments, seabass fed A-H and B-L diets tended to be more resilient to *Phdp*. Similarly, dietary treatments were able to delay disease progression after infection with *T. maritimum*.

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European seabass fed A-L dietary treatment showed higher plasma peroxidase levels compared to their counterparts fed B-L at 24 h following infection with *Phdp*, while the opposite pattern was observed after infection with *T. maritimum*, with lower plasma peroxidase activity in fish fed A-L compared to those fed the control diet. Hepatic oxidative stress biomarkers were not modulated by dietary treatment after infection with *Phdp*. However, an increase in lipids peroxidation was observed in seabass fed A-H compared to those fed the control diet after infection with *T. maritimum*. Gene expression data also showed a modulatory effect of dietary treatments after infection. In particular, European seabass fed the B-H diet showed highest transcripts of several immune-related genes at local level (i.e., mucosal tissues) after infection with *T. maritimum*.

**Conclusion**

Dietary supplementation of *E. gracilis* bioactive compounds seems to modulate immune and oxidative stress responses in European seabass submitted to different infection models (i.e., intraperitoneal injection and bath inoculation). Data from the present study suggest great potential for the development of functional feeds using *E. gracilis* bioactive compounds. Further studies are required to investigate other feeding times as well as more diverse bioactive compounds concentration in aquafeeds.

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# ENHANCING STEM EDUCATION THROUGH XR-INTEGRATED AQUAPONICS: FINAL RESULTS FROM THE XRAqua PROJECT

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## Introduction

XRAqua is a pioneering educational project that merges real-world aquaponics systems with an interactive virtual reality (VR) application to enhance STEM education through immersive, data-driven experiences. The project addresses the urgent need for innovative pedagogical approaches linking sustainability, aquaculture, food production, and digital technologies in secondary education, while raising awareness of low-trophic aquaculture solutions.

## Methodology

Throughout the 2024/2025 school year, a fully operational freshwater aquaponics system was installed at Madeira Torres Secondary School (Portugal), incorporating the cultivation of fish species alongside hydroponic vegetable production in a closed-loop design. Students interacted with the physical system and its digital twin via a dedicated XR application, allowing them to monitor parameters (e.g., temperature, pH, dissolved oxygen, electrical conductivity) using IoT sensors connected to an interactive dashboard, perform maintenance simulations, and engage with gamified sustainability challenges. User acceptance, educational impact, and system usability were systematically assessed through surveys, quizzes, and system data analytics.

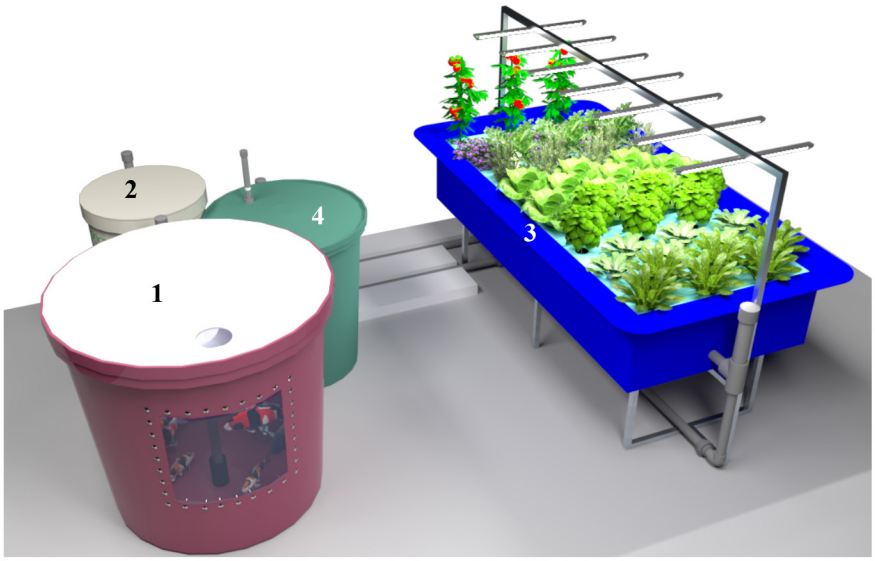


Fig. 1 - 3D perspective of the aquaponics system installed in the school, including: 1. 500 L fish tank, 2. Radial flow settler, 3. Hydroponic grow bed, and 4. Biofilter and water pump.

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**Results**

By September 2025, XRAqua will have completed its implementation phase, with comprehensive datasets gathered from more than 150 students across scientific, technological, and environmental disciplines. Preliminary findings demonstrate notable improvements in students' scientific literacy, critical thinking, and understanding of sustainable aquaculture practices. Over 85% of participants reported higher motivation towards STEM subjects, and significant increases were recorded in knowledge acquisition related to fish physiology, water quality management, and circular food systems. The XR platform achieved high usability ratings, and real-time data visualization via the online dashboard was pivotal in maintaining student engagement. Fish survival and growth rates in the pilot system were consistent with expected performance benchmarks for small-scale educational aquaponics setups, validating the technical feasibility of integrating aquaculture training into secondary school curricula.

**Conclusion**

XRAqua confirms the potential of combining XR technologies with aquaponics and fish farming as an effective educational strategy to foster sustainability awareness and build future competencies in aquaculture. The project's outcomes suggest that such hybrid learning models can be successfully scaled across educational settings, bridging gaps between theoretical knowledge, hands-on practice, and digital innovation, while contributing to broader blue economy and food security objectives.

# EFFECTS OF SINGLE AND COMBINED MICROALGAE ON LARVAL GROWTH, DEVELOPMENT AND SURVIVAL OF THE SEA CUCUMBER *Holothuria arguinensis*

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## Introduction

The overexploitation of wild sea cucumber stocks calls for sustainable aquaculture to meet demand and aid conservation (Purcell et al., 2012). *Holothuria arguinensis*, valued for its commercial potential and facing population declines, is a strong aquaculture candidate (Domínguez-Godino & González-Wangüemert, 2020). Production success depends on optimizing larval rearing, particularly microalgal diets with suitable nutritional profiles to support growth, development and survival (Domínguez-Godino & González-Wangüemert, 2019; Sibonga et al., 2021). In related species like *H. scabra* and *Apostichopus japonicus*, diets rich in protein, lipids, and essential fatty acids (EPA, DHA) have improved larval performance (Duy et al., 2016; Sibonga et al., 2021). Despite these advances, the role of microalgae such as *Rhodomonas* (Yamamoto et al., 2015) in *H. arguinensis* have not been thoroughly investigated, and understanding the link between algal composition and larval development is essential for refining hatchery protocols. This study aims to evaluate the effects of *Rhodomonas* sp. and *Isochrysis* sp. diets, on the growth, development, and survival of *H. arguinensis* larvae to contribute to the development of effective feeding strategies for its aquaculture.

## Material and methods

*Broodstock, larvae and microalgae diets*

Adult specimens of *H. arguinensis* were induced to spawn by thermal stimulation. At 24 h post-fertilization, larvae were stocked at 0.75 eggs/mL in 200 L conical tanks with 1 µm filtered, UV-sterilized seawater, renewed daily by 50%. Triplicate 50 mL samples were taken twice weekly for four weeks to monitor larval stages. Ten larvae per replicate were photographed upon stage transitions, with length and width measured using ImageJ Software. Larvae were fed daily with either a mixed diet of *Rhodomonas* sp. and *Isochrysis* sp. (5000 cells/mL each) or *Isochrysis* sp. alone (10,000 cells/mL). *Isochrysis* sp. microalgae were cultured in 5 L glass flasks and *Rhodomonas* sp. in 20 L polyethylene bags, both using sterilized seawater with F/2 medium (Guillard, 1975), under a 16:8 h light : dark cycle at room temperature. Cultures were harvested and counted daily using a Neubauer chamber.

*Biochemical analysis*

Proximate analysis of the microalgae was conducted using standard procedures (AOAC, 2023) and fatty acids analysis and identification was performed according to the protocols of Duy et al. (2016).

## Results and discussion

Unlike the findings of Duy et al. (2016) and Sibonga et al. (2021), no significant differences in auricularia larval length were observed across treatments from day 3 to 28 ($P > 0.05$). However, larvae were generally larger than those reported for *H. arguinensis* in previous studies (Domínguez-Godino & González-Wangüemert, 2019; Sousa et al., 2023). Late-stage auriculariae fed a mixed diet including *Rhodomonas* sp. were longer (1002.42 ± 148.59 µm) and significantly wider (744.31 ± 130.57 µm) than those fed *Isochrysis* sp. alone (981.71 ± 130.97 µm; 664.29 ± 116.88 µm). This may reflect the higher protein and EPA, and lower lipid content of *Rhodomonas* sp. (Tables 1 & 2), previously linked to enhanced larval performance (Duy et al., 2016). Its inclusion also significantly improved survival at days 14 and 21 (62.11 ± 3.65% and 41.33 ± 2.41%) compared to *Isochrysis* sp. (40.44 ± 2.91% and 26.66 ± 10.07%), and accelerated the appearance of the doliolaria and juvenile stages by 10 days.

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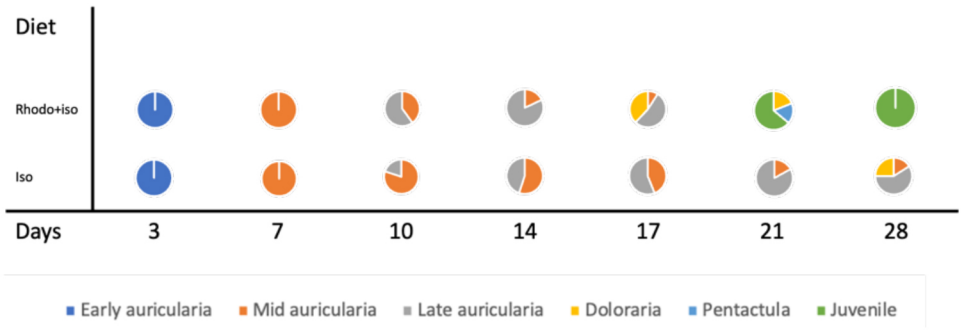


*Figure 1: Relative abundance (%) of each developmental stage of H. arguinensis at each sampling time post-fertilization according to different diets*

Table 1&2: Fatty acid composition (percent total fatty acid) and proximate biochemical analysis (percent dry weight) of the microalgae diets tested

| Name | *Rhodomonas* sp. | *Isochrysis* sp |
|---|---|---|
| Σ SFAs | 26.78 | 39.15 |
| 18:1n-9 Oleic | 1.78 | 10.96 |
| Σ MUFAs | 12.27 | 26.2 |
| 18:2n-6 Linoleic | 5.06 | 4.44 |
| 18:3n-3 Linolenic | 29.73 | 6.65 |
| 18:4n-3 | 12.57 | 15.34 |
| 20:4n-6 (ARA) | 0.23 | 0.18 |
| 20:4n-3 | 0.52 | 0.02 |
| 20:5n-3 (EPA) | 5.68 | 0.47 |
| 22:5n-3 (DPA) | 0.17 | 0.05 |
| 22:6n-3 (DHA) | 4.82 | 4.66 |
| Σ PUFAs | 60.94 | 34.64 |
| Σ n-3 PUFA | 54.24 | 27.62 |
| Σ n-6 PUFA | 5.93 | 6.37 |
| n-3/n-6 | 9.15 | 4.33 |

| | ***Rhodomonas* sp.** | ***Isochrysis* sp** |
|---|---|---|
| % Lipids | 16.32 ± 0.5 | 30.18 ± 0.31 |
| % Ash | 20.37 ± 0.13 | 16.81 ± 0.4 |
| % Proteins | 44.51 ± 1.48 | 36.71 ± 0.37 |
| % Carbo | 18.79 ± 1.76 | 16.3 ± 0.28 |

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# PROTEOMIC CHARACTERIZATION OF SMOLTIFICATION IN ATLANTIC SALMON (*Salmo salar*) REVEALS SEQUENTIAL MOLECULAR ADAPTATIONS DURING SEAWATER TRANSITION

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**Introduction**
Smoltification represents a critical developmental transition in Atlantic salmon (*Salmo salar*) aquaculture, with successful adaptation to seawater directly impacting production outcomes. Despite its importance, the molecular mechanisms underlying this complex physiological transformation remain incompletely understood. This study employed a comprehensive proteomic analysis to elucidate the temporal progression of protein expression changes during smoltification to inform targeted nutritional strategies.

**Methods**
Blood samples were collected from a single cohort of Atlantic salmon at four developmental stages: pre-smolt, smolt, 3-week post-smolt, and 3-month post-smolt. Proteomic analysis identified ~ 3,500 proteins per sample, with 2,588 proteins retained for differential expression analysis. Principal Component Analysis (PCA) was performed to distinguish developmental stages based on proteome signatures. Weighted correlation network analysis (WGCNA) identified protein modules with stage-specific expression patterns. Gene Ontology (GO) and KEGG pathway enrichment analyses characterised the biological processes and pathways altered during each developmental transition.

**Results**
PCA revealed distinct proteome signatures for each developmental stage, with PC1 (11.1%) primarily separating pre-smolt/smolt from post-smolt stages (Fig. 1). WGCNA identified seven distinct protein modules with stage-specific expression patterns showing moderate to weak correlations (-0.16 to 0.12) with developmental stages, indicating complex regulatory networks underlying the smoltification process. Six modules demonstrated highly significant ($p < 0.001$) differences in expression across developmental stages.

The pre-smolt to smolt transition was characterised by extensive transcriptional reprogramming, with significant enrichment of RNA splicing (FDR < 1.0e-45) and spliceosome pathways (FDR < 1.0e-40). This stage also showed activation of necroptosis and NOD-like receptor signalling pathways, indicating immune system involvement in initial adaptation. The MEgreen module exhibited the highest expression in smolt stage (0.122 ±0.077), suggesting involvement in smoltification-specific processes.

The smolt to 3-week post-smolt period revealed marked upregulation of translation (FDR < 1.0e-64) and ribosomal processes (FDR < 1.0e-85), indicating intensive protein synthesis. This transition was characterised by significant enrichment of carbon metabolism, oxidative phosphorylation, and tight junction pathways essential for osmotic regulation. The MEbrown module peaked at the 3-week post-transfer stage (0.095 ±0.022), showing inverse correlations between pre-smolt (-0.15) and post-transition stages, suggesting involvement in seawater adaptation processes.

The final transition to 3-month post-smolt involved upregulation of protein quality control mechanisms, including protein folding and regulation of proteolysis (FDR < 1.0e-11). Phagosome pathways showed the highest enrichment (FDR < 1.0e-11), alongside necroptosis and protein processing in the endoplasmic reticulum. The MEred and MEyellow modules showed the strongest positive correlation with the 3-month post-transition samples (0.12), indicating involvement in long-term seawater adaptation mechanisms.

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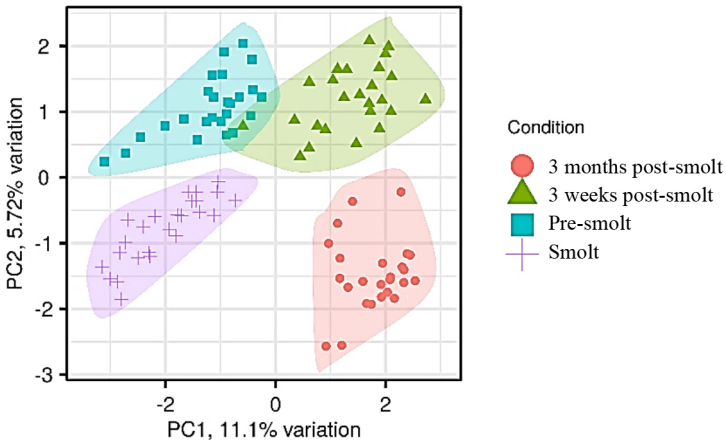


**Fig 1.** Principal Component plot of the blood proteome samples from the four stages of smoltification. Separation is most pronounced along PC1, with the post-smolt on the positive side and pre-smolt and smolt on the negative side.

**Discussion**

This study reveals that smoltification requires substantial energetic investment and precise protein regulation, evidenced by coordinated expression of energy metabolism modules (MEyellow, MEred) and protein homeostasis modules (MEturquoise, MEgreen, MEbrown) across different developmental stages. The significant upregulation of translation and ribosomal processes during the smolt to post-smolt transition suggests this period represents a critical window where nutritional support for protein synthesis could enhance adaptation success. These findings align with previous studies reporting increased protein synthesis in Atlantic salmon during smoltification but provide a more comprehensive temporal characterisation of the process.

The concurrent activation of immune-related processes throughout smoltification, particularly complement activation and NOD-like receptor signalling pathways, suggests that immune system modulation is an integral component of the adaptation process rather than a secondary response. This finding has important implications for feed formulation, suggesting that immunostimulants or immune-supporting nutrients might be beneficial during smoltification. The enrichment of tight junction pathways during seawater transition further emphasises the importance of intestinal barrier function in osmotic adaptation, pointing to potential benefits of dietary components that support epithelial integrity. Additionally, the enrichment of cellular detoxification and stress response pathways in later stages suggests potential benefits from antioxidant supplementation during long-term seawater adaptation.

This proteomic analysis provides a detailed molecular framework for understanding smoltification in Atlantic salmon, highlighting critical periods of metabolic demand and protein synthesis that could be targeted with specific nutritional strategies. These findings may inform the development of stage-specific feed formulations to support the substantial energetic and metabolic requirements revealed during the smoltification process, potentially improving production outcomes in salmon aquaculture.

# SEX-RATIO AND SEXUAL DIMORPHISM IN THREE NATURAL POPULATIONS OF EUROPEAN SEABASS *(Dicentrarchus labrax)* EXPOSED TO FOUR THERMAL REGIMES

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**Introduction**

With global warming, the surface temperature of the Mediterranean Sea has increased by 0.4 °C per decade from 1985 to 2006 (Nykjaer, 2009) and is projected to increase further by 2.2 to 3.4 °C until 2080 (IPCC, 2021). The European seabass, *Dicentrarchus labrax*, is very important for both aquaculture and fisheries in the Mediterranean. One of the big challenges in seabass farming is to improve the sex ratio in favour of the females, which grow faster and mature later. Moreover, seabass has a polygenic sex determination system with environmental influences, mainly driven by temperature (Vandeputte and Piferrer, 2019). Therefore, studying the impact of changing temperature on sex ratio is crucial for seabass production. In this study, we investigated the sex ratio and sexual dimorphism of three natural populations: Atlantic (AT), Western Mediterranean (WM) and Eastern Mediterranean (EM) in different thermal regimes.

**Materials and Methods**

The production of the three populations was achieved using three different full factorials with 30 sires per population and 7, 14 and 11 dams for the AT, WM and EM populations, respectively. Until 20 days post-hatching (dph), the populations were reared separately at 13°C in 2 replicate tanks per population. From 20 dph, the populations were mixed in equal proportions and placed in four thermal regimes that mimic the temperatures encountered in the Atlantic (*rAT*, annual average = 13.8 °C, min = 10.4 °C, max = 18 °C), the western Mediterranean Sea (*rWM*, annual average = 16.6 °C, min = 12.6 °C, max = 22.5 °C), the eastern Mediterranean Sea (*rEM*, annual average = 21.7 °C, min = 16.8 °C, max = 27.6 °C) and a temperature favouring females applied in aquaculture (*rAQUA*, 16 °C until 70 dph, 23 °C from 70 to 120 dph and then an eastern Mediterranean temperature regime). At a weight of 10 g, 6230 fish were tagged, weighed and genotyped with the Axiom™ Seabass 57k SNP DlabChip array. To recover the pedigree and population of each fish, parentage assignment was performed by exclusion using a subset of 1000 markers with the APIS R package (Roche et al., 2024)key issues are genotyping errors and an absence of parent genotypes because of sampling, traceability or genotyping problems. Most likelihood-based parentage assignment software programs require a priori estimates of genotyping errors and the proportion of missing parents to set up meaningful assignment decision rules. We present here the R package APIS, which can assign offspring to their parents without any prior information other than the offspring and parental genotypes, and a user-defined, acceptable error rate among assigned offspring. Assignment decision rules use the distributions of average Mendelian transmission probabilities, which enable estimates of the proportion of offspring with missing parental genotypes. APIS has been compared to other software (CERVUS, VITASSIGN, with two allelic mismatches tolerated. Each fish was sexed by macroscopic examination of the gonads at slaughter which occurred between 1 and 3 years of age in different fish groups. Threshold genomic models using the blupf90 suite of programs and the genomic relationship matrix were used to estimate heritabilities and genetic correlations, and to assess genotype-by-environment interaction (GxE) of sex between regimes.

**Results**

5704 fish were unambiguously assigned to their parents and sexed as a male or a female. A significant interaction was observed between thermal regime and population on sex ratio. For each population, significantly less females were observed in *rAT* than in the other regimes ($P < 1e\text{-}04$). For the WM population, significantly less females were observed in *rWM* than in *rAQUA* ($P < 1e\text{-}04$). In each thermal regime, there were more females in the AT population compared to WM and EM. For the warmer regimes (*rEM* and *rAQUA*), all populations were significantly different from each other, and WM had more females than the EM population (Fig. 1). Globally, the sex ratios of the AT population were balanced with an average of 46.9 % of females whereas the ones for Mediterranean populations were biased toward males, even more in colder regimes (*rAT* and *rWM*).

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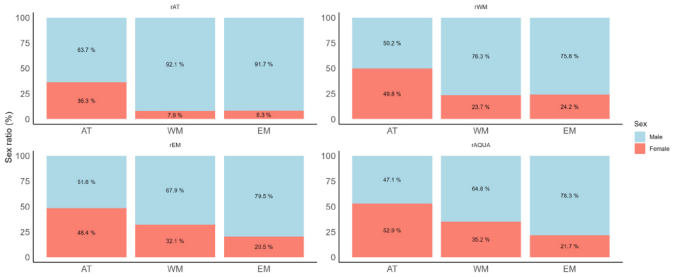


Fig. 1 - Sex ratio of three populations (AT = Atlantic, WM = Western and EM = Eastern Mediterranean) of European seabass in four thermal regimes (rAT = Atlantic, rWM = West-Med, rEM = East-Med and rAQUA = aquaculture regimes) since 20 dph.

A 3-way interaction between regime, population and sex was significant for body weight at the time of tagging (≈ 10 g, P = 1.19e-04), meaning that the sex dimorphism differed among populations and thermal regimes. In colder regimes (*rAT* and *rWM*), no interaction was found between sex and population and females were 28.5 % heavier than males on average. In the warmer regimes, the interaction between sex and population was significant (P < 8.08e-04). The difference in weight between females and males was stronger for the AT population (> 50 %) compared to Mediterranean populations (33-42 %). The heritability of sex was similar between AT and EM populations, averaging 0.64 ± 0.07. Further analyses will be presented, especially regarding GxE for sex-ratio..

## Discussion

The results confirmed that the sex of European seabass is strongly influenced by temperature but also has a strong genetic basis, showing for the first time a population effect with the AT population producing more females. We also showed that the sexual size dimorphism of body weight in favour of females depended on population and thermal regime with higher sexual dimorphism of body weight for the AT population in warmer regimes (*rEM* and *rAQUA*). Globally, we decrypted two groups of thermal regimes with one represented by *rAT* having temperatures below 13 °C until 50 dph and one represented the other thermal regimes. Indeed, the *rAT* had sex ratio more biased toward males than the other thermal regimes for the three populations and confirmed that a long lasting of cold rearing temperature promote the proportion of males (Vandeputte et al., 2020). Further analyses will describe why AT population expressed higher proportion of females with the results of heritability and genetic correlations but also studying the sex determination QTLs between the populations. This study is part of FishNess project (ANR-21-CE20-0043).

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# EUROPEAN SEA BASS BETA-DEFENSINS EXERT DIRECT ANTIVIRAL AND ANTIBACTERIAL FUNCTIONS

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**Introduction**

Aquaculture boom brings about new challenges such as bacterial and viral outbreaks. In this context, finding new molecules with no negative impact for the host neither the environment become an urgent issue. Antimicrobial peptides (AMPs) have arisen as potential candidates as treatments in aquaculture due to their properties and functions (Katzenback, 2015). Focusing on their direct role against pathogens, AMPs possess great bioactivity against a wide range of microbes including bacteria and viruses with relevance in mariculture. Among AMPs, we can find beta-defensin (Bdef) peptides, which have demonstrated antibacterial and antiviral activity in several species (Das et al., 2022). However, these roles for Bdef derived from European sea bass (*Dicentrarchus labrax*) have not been evaluated yet. Thus, the objective of this work was to assess the antiviral and antibacterial activities of (Bdef1 and Bdef2 derived from European sea bass, reduced or oxidated, against the most relevant viruses and bacteria for aquaculture.

**Material and methods**

European sea bass Bdef1 (4903 and 4904) and Bdef2 (4905 and 4906) mature peptides were synthesized by solid phase multiple peptide system using the Fmoc/tBu strategy (Houghten, 1985). Peptides were lyophilized and analyzed by MALDI-TOF mass spectrometry. The antibacterial activity of the four synthetized peptides was determined by evaluating the bacterial growth curves of *Vibrio harveyi*, *Vibrio anguillarum* and *Photobacterium damselae* using a method that was previously described (Sunyer & Tort, 1995). Bacteria was incubated with different dilutions of each peptide (0.6, 2.5 and 10 μg/mL). Data were expressed as the % of bacterial growth inhibition. Otherwise, the antiviral activity of the four synthetized peptides against RGNNV, SJNNV/RGNNV, RGNNV/SJNNV, EVEX, EVE, IPNV, SVCV and VHSV was performed following a previously described method with modifications (León et al., 2020). E-11 or EPC cells were seeded in 96-well plates until the 60-80% confluence. Equal volumes of different viral stocks were incubated with 500 μg/mL of the peptide during 24 h at the optimal temperature of each virus.

**Results**

The antibacterial effects of Bdef differed according to the tested bacteria. Inhibition rates of *V. harveyi* increased with the doses of reduced Bdef peptides whilst were decreased for oxidized ones. Otherwise, reduced Bdef1 or oxidated Bdef2 exerted the greatest growth inhibition against *P. damselae*. Contrary to this, for *V. anguillarum*, the activity for all peptides was very low and, in some cases, undetectable.

The oxidized Bdef peptides showed similar or higher antiviral activity. Also, differences between both Bdef1 and Bdef2 were observed. For instance, Bdef1 showed highest activity against RGNNV, SJNNV/RGNNV, IPNV, SVCV and EVEX, followed by VHSV, with no activity against EVE, while Bdef2 peptides exerted highest antiviral activity than Bdef1 against VHSV and EVE but lowest against RGNNV, SJNNV/RGNNV, IPNV, SVCV and EVEX. Surprisingly, RGNNV/SJNNV and TiLV were completely resistant to both Bdef peptides.

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**Discussion and conclusions**

Since oxidation process to establish disulfide bonds in peptides is so difficult, in this project we evaluated the differences between oxidated and reduced forms of Bdef peptides. The benefits of generating reduced transcripts includes reduced costs and faster synthesis but the alteration in secondary structure must be considered (Yang et al.,2017). Bdef disulfide bridges are tightly related to their bioactivity and the previous studies have not reach a clear conclusion yet (Schroeder et al., 2011; Yang et al., 2017). Our results from the antiviral trials pointed to oxidated peptides as more potent antiviral molecules in most cases while data from antibacterial assays revealed that oxidated peptides were more effective in lower doses than reduced peptides. Therefore, oxidation process might be necessary for European sea bass to be bioactive. Otherwise, when comparing the effects of Bdef1 on the bacterial growth or viral replication only were higher than those derived from Bdef2. In conclusion, European sea bass Bdef1 oxidated peptides could be postulated as potential tools to prevent or treat bacterial and viral infections in European sea bass farms.

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# POLYCHAETES FED WITH FISH BY-PRODUCTS SHOWED ANTIBACTERIAL RESISTANCE AND PROMISING APPLICATIONS IN AQUACULTURE

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**Introduction**

In the pursuit of sustainable solutions for aquaculture, there is increasing interest in identifying natural sources and their potential applications. Polychaetes, which are attracting great environmental attention, have arisen as potential solutions in aquaculture in two ways: they could be used as bioremediation agents of the aquaculture by-products as well as alternative fish and crustacean food sources (Pombo *et al*. 2018; Wang *et al*. 2019; Yang *et al*. 2022). In addition, their feasibility to grow in rich microbial environments and their ecological adaptability suggest they may harbour potent innate immune activities (Berlov and Maltseva 2016) such as antimicrobial peptides and other immune effectors (Joo *et al*. 2020). These activities could enhance, by one side, the removal of fish pathogens in aquaculture, and by the other, increasing the nutritional and immunological value when used as functional feed ingredients.

**Material and methods**

Juvenile *Perinereis vallata* polychaetes underwent a 35-day feeding with a commercial diet (Control) and three experimental groups fed with at 5%, 10%, and 20% Atlantic salmon sludge (Salmones Austral S.A.) as elsewhere (Gómez *et al*. 2023). All specimens were dehydrated at 60 °C, homogenised in PBS (pH 7.2) and supernatants obtained by centrifugation. Their immune and antimicrobial capacity were determined (León *et al.* 2020; Valero *et al*. 2023). Data were represented as the mean per group ± standard error of the mean ($n = 3$) and significance analysed by Student t test ($p < 0.05$) comparing experimental with Control group.

**Results and discussion**

A previous study already demonstrated that *Perinereis sp.* fed with salmon sludge increased their growth, survival, nutrient assimilation and removed total organic matter (Gómez *et al*. 2023). Our findings revealed that control polychaetes exhibited marked protease, antiprotease and antibacterial activities, effectively inhibiting the growth of several bacterial species. Specifically, control extracts suppressed bacterial proliferation by approximately 45-54% in *Vibrio harveyi* and *Aeromonas salmonicida*, and by 14% in *Photobacterium damselae*. In contrast, polychaetes fed with sludge, regardless of the proportion, showed a reduced ability to inhibit bacterial growth. Additionally, *Tenacibaculum maritimus*, an emerging salmonid pathogen recently reported in Chile (Apablaza *et al.* 2017), demonstrated enhanced growth when incubated with polychaete extracts. Additionally, we assessed the potential antiviral activity of *P. vallata* extracts.

**Conclusion**

This study demonstrates that polychaetes exhibit robust antiprotease, protease and direct antipathogenic activities, evidenced by their bacteriostatic effects against fish pathogens. These functions might be regulated by dietary inclusion of salmon sludge. Therefore, these polychaetes might be promising not only as bioremediation agents but also as natural sources of bioactive compounds for fish aquaculture. Their inclusion in fish diets may transfer immune effectors that enhance host immunity and resistance to infections. Thus, beyond their nutritive value, the immune-stimulating properties of polychaetes may provide an added functional benefit, reinforcing their potential as innovative antipathogenic and immunostimulant feed additives for sustainable aquaculture practices.

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# DIETARY MICROALGAE EXTRACTS AS FUNCTIONAL INGREDIENTS IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILE DIETS: EFFECTS ON IMMUNITY AND DISEASE RESISTANCE

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**Introduction**

The aquaculture industry is rapidly expanding, demanding effective disease prevention strategies. European seabass (*Dicentrarchus labrax)*, a key Mediterranean species, faces significant disease challenges (FAO, 2023). Functional feeds, particularly those incorporating microalgae, offer a promising and sustainable approach to enhance fish health and immunity. Microalgae such as *Tetraselmis chui* are rich in beneficial compounds including proteins, essential fatty acids, carotenoids, and other bioactive molecules, which contribute to improved growth, immune function, and disease resistance (Sansone et al., 2017). Additionally, other microalgae sources are known for their β-glucan content, further highlighting the diverse benefits of microalgae inclusion in aquafeeds. This study investigates the effects of dietary *T. chui* extracts and microalgae derived β-glucans on the immune response of European seabass and their resistance to a bacterial pathogen, after a short feeding boost of 5 and 14 days.

**Materials and methods**

European seabass juveniles (14.16 ± 1.01 g) were randomly assigned to triplicate tanks based on dietary treatments. Five experimental diets were formulated: a control diet based on a commercial formulation tailored to fulfill the dietary requirements of European seabass (CTRL), the control diet supplemented with a high dose of *T. chui* extract (Diet 1), the control diet supplemented with a low dose of *T. chui* extract (Diet 2), the control diet supplemented with microalga-derived β-glucans (Diet 3) and the control diet supplemented a commercially available product (CTRL+). Fish were fed the experimental diets for 5 and 14 days, after which they were sampled. At the end of each feeding period, the remaining fish were intraperitoneally injected with *Photobacterium damselae* subsp. *piscicida* strain MT1415 ($2.75 \times 10^5$ CFU/mL), and sampled 6 hours post infection. Samples from the gut, head-kidney, plasma, and liver were collected, in both sampling points. Mortalities were monitored over the following 8 days to assess disease resistance. In order to evaluate the effects of the dietary treatments and feeding period, a principal component analysis (PCA) followed by a canonical discriminant analysis (CDA) were employed, to obtain a systemic overview of the differential modulation, between feeding times and dietary treatments. Further analysis with ANOVA statistical test was employed at each sampling point and for each feeding time. The response to infection was also evaluated by a PCA and CDA. Mortalities were evaluated with Kaplan-Meier and Mantel-cox tests.

**Results and Discussion**

Feeding the animals for 5 and 14 days the experimental diets, generated a considerably different modulation of the immune status of European seabass, inciting the importance of carefully evaluating duration of feeding of functional feeds. Contrarily to previous observations by Reis et al. (2021), a short-term (5 days) supplementation with β-glucan (Diet 3) increased liver lipid peroxidation after infection. Meanwhile, this same diet applied for a longer period (14 days) led to lower cortisol levels and boosted key antioxidant enzymes, suggesting a later enhanced resilience to stress while improving oxidative status. The inclusion of *T. chui* extract showed a dose-dependent effect with the higher dosage (Diet 1) remarkably reducing gut lipid peroxidation in non-infected fish, highlighting its described potent antioxidant properties (Randazzo et al., 2023). In contrast, at lower dosage (Diet 2), the microalgae dietary inclusion, resulted in a less favourable oxidative stress profile and less noticeable immune modulation. Finally, diets and feeding duration did not significantly alter the survival of European seabass juveniles in response to the bacterial challenge.

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Altogether, these findings suggest that supplementing both *T. chui* extracts and β-glucans in European seabass diets for a short period of 14 days prior to an acute stress, may be a positive prophylactic strategy. The results underscore the importance of both dosage and feeding duration for optimizing the benefits of these functional ingredients in aquaculture.

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# SKIN MUCUS AS A NON-INVASIVE MATRIX TO ASSESS WELFARE INDICATORS IN FARMED GILTHEAD SEABREAM (*Sparus aurata*)

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**Introduction**

Aquaculture production has rapidly grown in recent years, accounting for 51% of the global fish production in 2022 (FAO, 2024). To meet the increasing demand for seafood, sustainable aquaculture practices represent a promising alternative to traditional systems, as they face several environmental and biological issues (Taşbozan, 2023). One of the most important challenges in aquaculture is ensuring fish welfare, which is typically assessed through a global set of parameters, including physiological indicators measured in blood and plasma, which are among the most widely used. However, procedures such as blood sampling are invasive and may provoke additional stress, potentially compromising the animal's well-being (Carbajal et al., 2019). In this context, skin mucus has been increasingly used as an alternative non-invasive method to assess fish welfare indicators. Yet, only a few studies were conducted on Gilthead seabream (*Sparus aurata)*, focusing on a limited range of parameters (Guardiola et al., 2016; Fernández-Alacid et al., 2018). Moreover, limited attention has been given to correlations and temporal dynamics between parameters measured in both plasma and mucus matrices. Therefore, this study aims to determine whether skin mucus can be used as a valid non-invasive alternative to plasma for welfare assessment in farmed Gilthead seabream, by evaluating both mucus-plasma correlations and temporal trends of a set of six physiological indicators (i.e. glucose, lactate, cortisol, total protein, albumin and IgM) related to stress and immunity response of the fish in these two biological matrices.

**Materials and methods**

All procedures involving animals were performed following Italian national legislation (D. lgs. 26/2014) and EU recommendation (Directive 2010/63/EU), with Health Ministry authorisation number code n° 522/2023-PR. Two commercial offshore Gilthead seabream farms located in northern Sardinia (Italy), hereafter referred to as sites A (organic farm) and B (conventional farm), were selected for this study. Fish (average weight: 380 ± 24 g; total n = 30) were sampled for plasma (blood sampling) and skin mucus at two time points after anesthesia: T0 (August 2024; n = 10 at site A) and T1 (May 2025; n = 10 per site). Prior to laboratory analysis, both blood and mucus samples were centrifuged to obtain plasma and mucus supernatant, respectively, which were both frozen at -20 °C until use. Glucose, lactate, cortisol, total protein, albumin and IgM were measured in both plasma and skin mucus using commercial kits, following the manufacturer's instructions. Statistical analyses were carried out using R software (version 4.5.0). Spearman correlation was used to evaluate the relationship between welfare indicators derived from the different matrices. The permutation-based linear model was used to assess temporal trends in glucose, lactate, cortisol, and total protein levels across plasma and skin mucus, as albumin and IgM were excluded from this analysis due to insufficient data at T0.

**Results**

Glucose, lactate, cortisol, total protein and albumin levels did not show a significant correlation between plasma and skin mucus samples. In contrast, a significant positive correlation was observed in IgM levels between skin mucus and plasma ($r = 0.558$; $p = 0.009$). The results of the permutation-based linear model showed a significant Matrix × Time interaction ($p < 0.05$) for glucose, lactate and cortisol contents, indicating different temporal dynamics between plasma and skin mucus. These physiological indicators remained constant over time in skin mucus, while changes were observed in plasma. Particularly, plasma lactate levels approximately doubled over time, while glucose and cortisol contents in plasma decreased by about 45% and 89% between T0 and T1, respectively. In contrast, no significant Matrix × Time interaction ($p > 0.05$) was observed for total protein, suggesting a similar temporal trend between both biological matrices. Specifically, total protein in T1 increased by about 21% from T0, regardless of the biological matrix.

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**Discussions**

Although total protein levels showed a similar temporal trend in both plasma and skin mucus, no significant correlation was found between these two biological matrices. This contrasts with the findings of Fernández-Alacid et al. (2019), who reported a significant correlation between plasma and skin mucus protein levels in meagre (*Argyrosomus regius*), suggesting potential species-specific differences or limited statistical power due to sample size. In fact, a significant positive correlation was found in IgM levels between plasma and skin mucus, indicating similarity in systemic and mucosal physiological dynamics. However, no similar temporal trends or mucus-plasma correlations were found in glucose, lactate and cortisol levels, contrasting with the findings of Fernández-Alacid et al. (2019) in meagre and thus supporting species-specific differences. As hydrophilic molecules, the stability of glucose and lactate over time may indicate their dispersion within the surrounding water, while the differences observed for cortisol support the findings of Guardiola et al. (2016), who reported a delay between the measurement of plasma and skin mucus cortisol in Gilthead seabream, suggesting different systemic and mucosal physiological dynamics.

**Conclusion**

These results suggest that skin mucus can be used as a potential non-invasive proxy to assess some particular welfare indicators (i.e. total protein, IgM) in farmed Gilthead seabream. However, the set of parameters evaluable in skin mucus is limited compared to those in plasma. Therefore, further studies with larger sample sizes, as well as exploration of physiological mechanisms behind molecular transfer between plasma and skin mucus, and the analysis of additional indicators, are needed to support these preliminary findings.

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# SALMON LICE DISLODGED IN DELOUSING OF FARMED SALMON CAN SURVIVE AND REATTACH TO NEW FISH

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**Introduction**: Sea cages used in Atlantic salmon aquaculture are open to the environment, leading to infestation by the naturally occurring parasite the salmon louse (*Lepeophtheirus salmonis*). Salmon lice infect the skin of the salmon and delousing treatments to reduce parasite spillover to wild salmon populations dislodges the mobile (preadult and adult) stages of the parasite. Depending on the method and procedures, this can lead to the release of large amounts of lice to the surrounding water. In a natural environment, salmon lice are dispersed to other fish as copepodites, and the question remains whether these later mobile stages of the parasite can survive and spread to new fish?

**Methods**: Dislodged mobile lice were monitored in the laboratory for survival at different temperatures and salinities and ability to attach to new fish. Furthermore, the sinking rates of lice were investigated experimentally in tanks and open water. These results were utilized in a hydrodynamic dispersal model to quantify potential spread to other farm sites.

**Results and discussion**: All mobile lice exhibited long survival off fish with most lice surviving more than one week. Warmer temperatures and lower salinity reduced overall survival. Adult females survived longer than preadults and adult males. Also, lice reattachment to new fish was only slightly reduced even by prolonged removal from the host fish. Whereas the long survival times and high rates of reattachment highlight the risk of reinfestation time, the mobile lice exhibited limited ability to remain in the water column. The rate of sinking was approximately 1metre per minute with the larger adult females sinking faster than the smaller stages. Adult males appeared as stronger swimmers reacting to host cues in the water. Accordingly, modelled dispersal indicates that the highest risk of reinfestation is within farms and to wild fish in the vicinity of cages. Farms in the surrounding area have a much lower risk, but delousing under circumstances with significant water transport to farms downstream should be avoided.

# INITIAL INFESTATIONS OF SEA LICE ON MIGRATING ATLANTIC SALMON POST SMOLT ARE DOMINATED BY *Lepeophtheirus salmonis*

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## Introduction

Sea lice (*Caligidae*) are marine ectoparasites of fish that feed on the skin, mucus, and in some species the blood of their host. At high densities, sea lice pose a major health risk to wild and cultured fish. The Atlantic salmon (*Salmo salar*) is a host to *Lepeophtheirus. salmonis* a sea louse species specific to salmonids and *Caligus elongatus* with a very broad host range. Production of *L. salmonis* on farmed fish is heavily regulated in Norway due to parasite spillover to the protected wild Atlantic salmon. Specifically, wild salmonoids are surveilled yearly to assess the threat of *L. salmonis*. Early stages of the two sea lice species (sessile stages) are difficult to distinguish morphologically resulting in unknown contribution by the two species. The purpose of the study was to develop a method to assign species in sessile sea lice and to determine the prevalence of the two species on wild post smolt.

## Methods

Migrating wild salmon which had recently left the freshwater habitat were caught in the outer part of fjords in six locations in Central and Western Norway. Three fjords were sampled for two years resulting in a total of nine samplings. Lice were counted and for the larger mobile stages (preadult and adult) were identified to the species level. For the smaller sessile stages (copepodites and chalimi), lice were sampled in ethanol for subsequent DNA extraction. In the laboratory, sessile lice (n= 1247) were identified as *C elongatus* and *L. salmonis* based on a boiling of samples followed by a species-specific PCR assay. 90% of the samples could be identified using this high-throughput methodology.

## Results and discussion

Given the relatively short timeline between entering their marine phase and when fish were caught, the vast majority (97%) of recorded lice were still sessile. The lice community was heavily dominated by *L. salmonis* which constituted 98 % of all identified sessile lice. The contribution of *C. elongatus* was very low and only exceeded 3 % in two samplings, which also had the overall lowest prevalence and mean abundance of lice. Mobile sea lice were only observed on 6 % of the sampled fish. Of the mobile lice (n= 415), 73 % were identified as *L. salmonis*, and 27 % as *C. elongatus*. Almost all mobile *C. elongatus* came from fish collected in Sognefjorden and Boknafjorden and was similar in both years sampled; 2023 and 2024. In accordance with the life strategy of *C. elongatus* utilizing multiple hosts, we observe a larger proportion of mobile lice of this species. Interestingly, the occurrence of *C. elongatus* appeared to be restricted to two out of six areas investigated in the time period investigated. Overall, we conclude that the sea lice community on migrating Atlantic post smolt in the investigated areas is dominated by sessile lice and that almost all of these lice are *L.salmonis*.

# RUMINAL FERMENTATION AS A BIOTECHNOLOGICAL STRATEGY TO GENERATE PROTEIN INPUTS FOR AQUACULTURE

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This study focuses on the development of biotechnological strategies aimed at obtaining alternative ingredients for fish nutrition. The objective was to generate a microbial protein extract from microorganisms in the sheep rumen. To this end, ruminal fluid was inoculated into flasks containing lignocellulosic residues of wheat straw under anaerobic fermentation conditions. The dynamics of bacterial and eukaryotic communities were analyzed before and after the fermentation process. Three ruminal fluid samples and three post-fermentation samples were analyzed using metabarcoding techniques, amplifying the V3-V4 regions of the 16S gene and V9 of the 18S gene. Sequencing was performed on the Illumina MiSeq platform. Bacterial (16S) analyses revealed a high initial predominance of the Bacteroidetes phylum (~70%), followed by Firmicutes (~25%). After fermentation, a notable reduction in the genus Prevotella (from ~60.2% to ~27%) and a significant increase in genera such as Lysinibacillus (from 2% to 22%), Herbinix (from 0.3% to 50%) and Proteiniborus (from 0% to 12.5%) were observed, suggesting a functional adaptation towards lignocellulose degradation and the production of protein-rich microbial biomass. Regarding eukaryotes (18S), an initial dominance of the phylum Ciliophora (~91%) was observed, especially of the genus Entodinium, whose abundance remained stable after fermentation. However, the appearance of representatives of the phylum Oomycota, such as Phytophthora and Peronospora, was detected, indicating a functional diversification possibly associated with the availability of lignocellulose-derived compounds. These results demonstrate that the fermentation of lignocellulosic waste with ruminal microbiota induces significant changes in the microbial structure, favoring the proliferation of taxa with the potential to produce high-protein biomass. This biomass represents a promising candidate as an alternative ingredient for evaluation in aquaculture diets.

# AN INSIGHT INTO FUNGAL COMMUNITY DIVERSITY, ABUNDANCE AND COMPOSITION OF REARING WATER AND RED TILAPIA GILL FROM OPEN RIVER CAGES AND EARTHEN PONDS BY APPLYING INTERNAL TRANSCRIBED SPACER RIBOSOMAL RNA AMPLICON METAGENOMIC SEQUENCING

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## Introduction

Aquaculture is crucial in tackling global food security issues where demand for food and economic growth is increasing. Tilapia, the world's second-largest farmed fish, plays a vital role in meeting global demands, particularly in Thailand. Integrating knowledge of mycobiome dynamics with environmental management and aquaculture operations can enhance fish health, water quality, and overall ecosystem stability. It also boosts feed efficiency and lessens the reliance on antibiotics, making this holistic approach essential for sustainable and productive aquaculture systems. While mycobiome research in aquaculture shows great promise, several limitations hinder a complete understanding and practical application. Addressing these limitations requires advancements in technology, more robust experimental designs, and deeper functional investigations to fully grasp the mycobiome's role in aquaculture.

## Materials and methods

Hence, this study, as part of a broader series, aimed to examine the abundance, composition, and diversity of fungal communities in the rearing water and gills of red tilapia cultured in earthen ponds and open river cages in Central Thailand. This was achieved through comprehensive sampling and ITS rRNA gene sequencing, revealing associations and variations across different farming methods and locations..

## Results

Our findings demonstrated a diverse fungal community with a wide range of taxa, where the top 21 fungal families accounted for over 5% of the total reads. Notably, *Cladosporium*, *Aspergillus*, and *Fusarium* were identified, with *Cladosporium* being the most abundant across all samples. Additionally, differences in fungal abundance and diversity were observed between water and gill samples, with *Candida spp*. predominating in gill samples and *Rhodotorula spp*. prevailing in water samples. Canonical Correspondence Analysis (CCA) showed associations between fungal abundance and environmental variables. Alpha diversity analysis revealed significant differences between water and gill samples, with the sampling source exerting the greatest effect on fungal diversity. Furthermore, beta diversity analysis indicated substantial variations in fungal community composition across sampling locations and farming styles, underscoring the impact of environmental factors and aquaculture practices on fungal community dynamics.

## Conclusion

Overall, these findings offer valuable insights into the abundance, diversity, and distribution of fungal communities in red tilapia farms, emphasizing the need to consider fungal pathogens in aquaculture management strategies. Understanding the dynamics of these communities is crucial for mitigating disease risks, maintaining fish health, and ensuring food safety in aquaculture. Further research is necessary to clarify the specific mechanisms of fungal pathogenesis and the effects of environmental factors on fungal community dynamics in aquaculture settings.

# SEAWEEDS FED BLACK SOLDIER FLY LARVAE IN DIETS OF SMOLTIFYING ATLANTIC SALMON: EFFECTS ON THE INTESTINAL FLUID UPTAKE AND GROWTH PERFORMANCE

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## Introduction

Marine aquaculture side-streams such as seaweeds are gaining increased attention as alternative aquafeed ingredients for fish relevent amino acid profile, presence of long-chain polyunsaturated fatty acids (PUFAs) and micronutrients. However, the complex carbohydrate structures in plant-based ingredients make them challenging to use directly in the diets of carnivorous species. Larvae of insects, as the black soldier fly larvae (BSFL) on the other hand, have emerged as promising candidates for convertion and valorization of different side streams and wastes into valuable feed ingredients. Particularly interesting are marine side-streams that have the potential to increase the PUFA and other nutritional values of the feed. Although some studies have explored the valorization of seaweeds through BSFL, salmon feeding trials incorporating algea derived BSFL are still scarse, particularly during sensitive developmental stages like the smoltification.

Atlantic salmon smoltification represent a critical life stage, when the fish undergo profound physiological changes to prepare for seawater (SW). A key adaptation during this transition is the developemnt of intestine and the fluid transport capacity which enables effective water absorption, a crucial osmoregulatory step in marine environments. Proper intestinal development during smoltification is therefore essential for successful seawater acclimation. To date, no studies have specifically investigated the impact of marine side-stream derived insect-based diets on intestinal fluid transport development and growth performance in Atlantic salmon during smoltification. Therefore, the main aim of the present study was to investigate how side-streams from seaweed farming, valorized through BSFL influence intestinal development in Atlantic salmon smolts with a particular focus on intestinal fluid transport capacity.

## Materials and methods

Atlantic salmon smolts were produced at the Lerang Research Station, Norway (Skretting Aquaculture Innovation), using a winter light regime (12 hours light:12 hours darkness, 12:12) for 6 weeks, followed by continuous light (24:0) at 12 °C. During the winter light period in freshwater (FW), triplicate groups of parr (~35 g) were assigned either a control diet or one of two insect meal diets: BSFL fed on Ulva (*Ulva fenestrata*) or BSFL fed on Kelp (*Saccharina latissima*). Insect meal diets (5% inclusion) were formulated to partially replace fishmeal in the control diet. The feeding trial was conducted for 12 weeks. Fish were then transferred to full-strength SW at 504 degree-days (dd). During the SW period, all fish were fed the same commercial diet for six weeks. Sampling was conducted in FW at 0 dd, 336 dd, and 504 dd as well as at the final sampling in SW at 1008 dd. At each sampling point, fish body weight (g) and intestinal mucosal weight (mg) were measured. Plasma was analyzed for ion concentrations, osmolality, and hormone levels (including IGF-1 and cortisol). Intestinal fluid transport rate was measured using the non-everted gut sac method, and $Na^+/K^+$-ATPase (NKA) enzyme activity using a kinetic enzyme assay. The intestinal nutrient transport capacity was also determined using Ussing chamber techniques.

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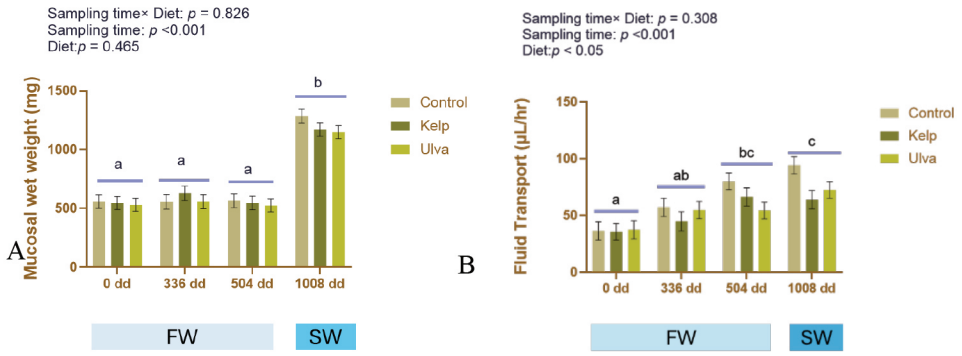


Figure 1: Intestinal mucosal mass (A) and fluid transport capacity (B). Lower case letters indicate the significant difference between sampling time ($p$ <0.05).

| | 0 dd | 504 dd | 1008 dd |
|---|---|---|---|
| Control | 76.18±1.28 | 110.57±1.60 | 145.82±2.00 |
| Kelp | 77.62±1.28 a | 112.63±1.57 b | 152.87±1.95 c |
| Ulva | 77.49±1.28 | 110.09±1.57 | 152.83±1.95 |

Table 1: Body weight (g) of Atlantic salmon parr to 6 weeks in SW
0 dd- Body weight (BW) of the parr, 504 dd- BW at the end of FW period and 1008 dd- BW at the end of SW period. Lower case letters indicate the significant difference between different sampling times ($p$ <0.05).

**Results**

Mucosal weight increased over time, with significantly higher mucosal mass observed in fish sampled after 6 weeks in SW at 1008 dd. No significant differences in mucosal mass were found between diets (Fig 1-A). Intestinal fluid transport capacity also increased over time with significantly higher values in SW fish. A general dietary effect was observed, where fish fed the control diet exhibited significantly higher intestinal fluid transport capacity compared to those fed BSFL meal based on kelp (Fig 1-B). Growth results showed that body weight increased by nearly 20% in the smolts at 504 dd compared to the parr at 0 dd. After six weeks in seawater (1008 dd) body weight increased by approximately 50% compared to the parr stage (Table 1). However, body weight was not significantly affected by different diets (Table 1).These findings suggest that BSFL-based diets supported similar growth performance to the control diet, with Ulva-based diet being the most promising candidate as fish fed the kelp-based BSFL diet showed lower intestinal fluid transport capacity. In-depth physiological results, including intestinal nutrient transport, barrier function, and NKA activity will be presented at the conference. Overall, these findings highlight both the potential and the challenges of using BSFL-valorized seaweeds diets to support intestinal development and seawater adaptation in Atlantic salmon smolts.

# AMPHIBIOUS TECHNOLOGIES FOR SUSTAINABLE AQUACULTURE: THE ROLE OF MAR (MULTIPURPOSE AMPHIBIOUS ROVER) IN COASTAL MONITORING, SAFETY, AND RESILIENCE

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The development of European aquaculture increasingly requires innovative technologies that can support environmental sustainability, operational safety, and social acceptance of production activities.

In this context, the MAR – Multipurpose Amphibious Rover (www.eco-mar.it) represents an advanced and versatile solution for operations in complex coastal environments such as lagoons, estuaries, and intertidal zones—areas that are often beyond the reach of traditional automation and control systems.

Designed to move across land, mud, and shallow water, MAR is a modular, fully electric amphibious mobile platform developed for civil, environmental, and infrastructure protection applications.

Its integration into the aquaculture sector opens new opportunities for smart management of farms and continuous ecosystem monitoring.

This contribution explores the potential applications of MAR in support of sustainable aquaculture, with specific reference to the coastal context of Valencia and other Mediterranean regions:

- Real-time environmental monitoring using onboard sensors (water quality, turbidity, microplastics, etc.);
- Surveillance and protection of aquaculture infrastructure against natural damage or unauthorized access;
- Low-impact logistical support for inspection and maintenance of cages, rafts, and piers;
- Technical training and outreach, offering a mobile and interactive platform for education and stakeholder engagement.

MAR contributes to the Blue Economy paradigm and aligns with the goals of the European Green Deal, offering a concrete solution to drive a digital and green transition in the aquaculture sector. Its adoption can strengthen engagement with local communities, increase transparency, and improve the public perception of aquaculture as a sector compatible with environmental integrity and socio-economic development.

The presentation aims at the creation of collaborative pathways with academic institutions and local producers, laying the groundwork for integrated pilot projects in the Mediterranean region.

# FEEDING IMMUNITY: EVALUATING FUNCTIONAL DIET EFFECTS IN RAINBOW TROUT (*Oncorhynchus mykiss*) DURING AN *A. salmonicida* OUTBREAK

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**Introduction**

The misuse or overuse of antibiotics has caused the onset of antimicrobial-resistant strains of *Aeromonas salmonicida* in fish and the aquatic environment. Different strategies have been proposed to boost fish resistance to *A. salmonicida,* one of the major pathogens of salmonids. Among the preventive approaches explored to increase natural resistance to disease in fish, probiotics, prebiotics, and immunostimulants have emerged (Minářová et al. 2021).

This study conceived an integrated approach to explore the potential immunomodulatory effects of a commercial feed, Protec (Skretting), which contains β-glucans, vitamins C and E, and zinc (Bulfon et al., 2019), and its ability to mitigate the mortality caused by a naturally occurring *A. salmonicida* outbreak in adult rainbow trout.

**Materials and Methods**

Adult rainbow trout (~300 g) were farmed in central Italy (Granica, RI). The animals were fed once a day with two commercial pelleted diets: 1) Optiline (Optiline, Skretting), used as a control diet, and 2) Protec (Protec, Skretting). Shortly after starting the commercial feeding trial, the farmer noticed a steady low-level mortality in both groups. After three weeks, 25 Optiline and 33 Protec fish were randomly caught using a fishing wade and immediately sacrificed for further examinations, as follows. External welfare indicators (Noble et al., 2022), biometric and autoptic indices were recorded. Peripheral blood was collected from the caudal vein and immediately transferred to heparinized tubes. The etiologic agent of the outbreak was detected by inoculating Tryptone Soy Agar (TSA) and Blood Agar (BA) plates with target organs (brain, kidney, and spleen). Identification of the isolates was performed phenotypically by MALDI-TOF (MALDI Biotyper, Bruker), while the subspecies was genotypically identified by INgene® q *Aeromonas salmonicida* (Gold Standard Diagnostics) through the StepOne Plus (Applied Biosystems). Blood cell count was assessed at 400X magnification under an Axioplan microscope (Zeiss), and innate immunity parameters (leukocyte viability, Reactive Oxygen Species (ROS) production, and phagocytosis activity) were evaluated by flow cytometry with the CytoFLEX instrument (Beckman Coulter) and analysed with Kaluza software v 2.1 (Beckman Coulter).

Cell counts were analysed using the Chi-square test, while the other parameters were tested using the non-parametric Mann-Whitney (Bonferroni-corrected $p$-value; $p < 0.05$ was considered significant). Statistical analysis was performed with the open-source software Past v. 4.14.

**Result and discussion**

After three weeks of the commercial feeding trial, no differences in either external welfare indicators or biometric parameters were observed between the Optiline and Protec groups. However, both groups showed Hepato-Somatic Index (HSI) and Spleen Somatic Index (SSI) values nearly twice the optimal range for rainbow trout (Nabi et al. 2022; Cardinaletti et al. 2022), suggesting that the mortality recorded could be related to the presence of an infectious agent. Mortality was significantly higher in the Optiline group compared to Protec ($p<0.001$), suggesting an improved immune competence in trout fed with an immunostimulant-rich diet (Redivo et al. 2023).

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The bacteriological culture examination of the target organs revealed the presence of systemic *A. salmonicida.* The subspecies *salmonicida* was detected in blood samples with no statistical differences in prevalence (28% Optiline *vs.* 21% Protec). However, the Optiline group displayed a significantly higher *A. salmonicida* gene copy number compared to the Protec group ($p<0.05$), suggesting a less efficient pathogen clearance in individuals fed with the Optiline diet. Consistently, trout fed with Protec showed a higher proportion of blood lymphocytes ($p<0.05$) and thrombocytes ($p<0.05$), and a lower number of neutrophils ($p<0.05$). Lymphocytosis has been related to greater recruitment and activation of specific immune cells during systemic defensive response against bacteria (Bulfon et al., 2019). Furthermore, a significantly lower number of leukocyte aggregates (leucoagglutination) was detected in the Protec group compared to the Optiline ($p<0.01$).

Leukocytes isolated from trout fed with Protec showed significantly higher viability ($p<0.01$), and lower apoptosis and death ($p<0.05$), paralleled by lower ROS production ($p<0.05$). They also exhibited a greater proportion of phagocytic ROS-producing live myeloid cells ($p<0.05$). Overall, these data suggested that the immunostimulants included may induce a greater pathogen clearance in trout leukocytes (Dalmo and Bøgwald 2008).

**Conclusion**

Although severe alterations have occurred in fish affected by the pathogen, regardless of the diet administered, the data obtained by the conceived integrated approach suggest that the immunostimulant-enriched diet strengthens rainbow trout's immune systems, partially mitigating *A. salmonicida* infection.

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# DIETARY INCLUSION OF A NUTRITIONAL EMULSIFIER BASED ON POLYETHYLENE GLYCOL RICINOLEATE LOWERS THE NEGATIVE EFFECTS OF HEAT STRESS IN RAINBOW TROUT

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## Introduction

Climate change brings a significant challenge to aquaculture systems, especially in cage farming where rising water temperatures can negatively affect fish health and performance. Increasing water temperatures over the past decades have been reported for the Mediterranean Sea (Pastor et al., 2020), inland waters like the Black Sea in Turkey (Sakalli & Başusta, 2018), and lakes and rivers in Italy (Gavioli et al., 2024). Global warming is not expected to halt in the near future and will increasingly affect aquaculture. In rainbow trout (*Oncorhynchus mykiss*), heat stress can impair immune function and gut health, and reduce nutrient digestibility. The associated decline in performance poses a challenge for the productivity in aquaculture systems. With the dietary supplementation of a nutritional emulsifier based on polyethylene glycol ricinoleate (PEGR), an improved digestion, particularly of fats and oils, can be obtained. Moreover, our previous research already demonstrated microbiota shifts due to extreme heat in farmed gilthead sea bream were partially reversed by nutritional intervention with PEGR (Domingo-Breton et al., 2024). This study, evaluated the potential of a PEGR-based nutritional emulsifier to mitigate the effects of heat stress in rainbow trout.

## Material and Methods

A 12-week feeding study was conducted at the trial facilities of ABT Innovia in Malta with a total of 600 rainbow trout (initial weight 111.77g). Fish were randomly assigned to twelve tanks across four dietary treatment groups: Control, 750 ppm PEGR-based nutritional emulsifier (Volamel Extra produced by Nukamel BV, the Netherlands - 750V), 1000 ppm nutritional emulsifier (1000V) and 1250 ppm nutritional emulsifier (1250V). Fish were fed their respective experimental feeds as 4.5 mm pellets three times daily, at a fixed rate based on body size. Water temperature was maintained at 16 ± 2°C during the first eight weeks of the trial, and then gradually increased by 1°C per day to a final temperature of 22 ± 2°C to induce heat stress. Growth performance and health parameters were monitored and statistically analyzed with ANOVA, using post-hoc multiple comparisons with Tukey HSD test.

## Results

No significant differences between treatments were observed in the thermoneutral phase during the first eight weeks (Figure 1).

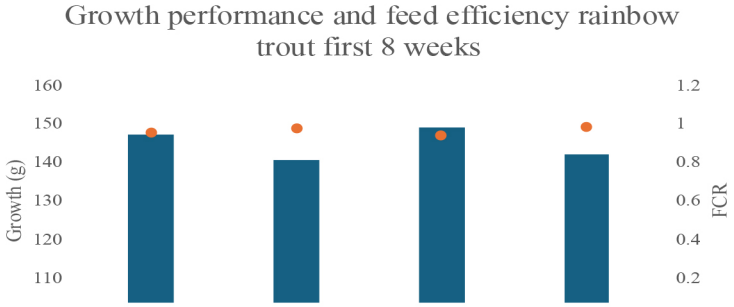


Figure 1: Growth performance and feed efficiency of rainbow trout fed diets with different levels of a PEGR-based nutritional emulsifier in thermoneutral condition

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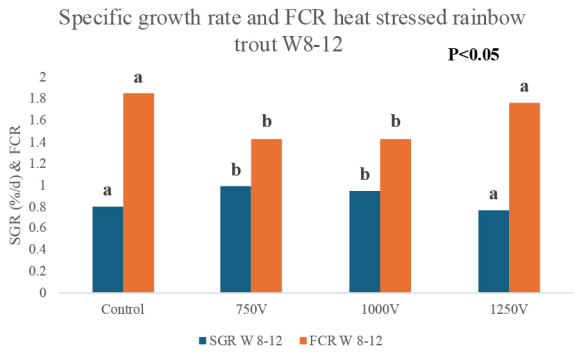


Figure 2: Specific growth rate and FCR of rainbow trout under heat stress from week 8 to week 12.

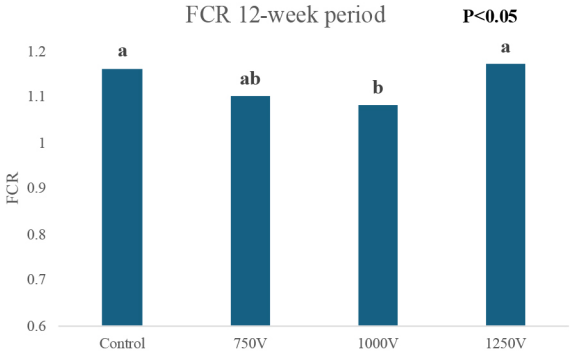


Figure 3: FCR rainbow trout fed different levels of emulsifiers over a 12week period

Under heat stress, both treatments with nutritional emulsifier (750V and 1000V) showed significantly higher growth and lower FCR compared to control (Figure 2). Survival was numerically better, while hepatosomatic index (HSI), and viscerosomatic index (VSI) showed a significant increase in the diets with the nutritional emulsifier.

For the overall 12 weeks trial period, 1000 ppm of nutritional emulsifier showed a significant improvement in the FCR ($p<0.05$) and a trend towards higher growth ($p=0.1$). Survival and K-factor (condition factor) were unaffected across treatments.

**Discussion**

During heat stress a lot of changes in metabolism take place and can be accompanied by changed levels of HSI and VSI and can be harmful for fish health. A decrease in performance is often recorded, resulting in production losses for aquaculture systems. Nutritional emulsifiers based on PEGR can influence the gut dynamics of carnivore fish. This trial showed that addition of a nutritional emulsifier can mitigate the negative effects on growth performance and feed efficiency of carnivore fish under heat stress challenge. More research is needed to see the effect on other mechanisms in the fish, such as digestibility, immunity and antioxidant capacity.

**Conclusion**

Inclusion of a nutritional emulsifier based on PEGR helped maintain growth and feed efficiency under heat stress conditions in rainbow trout. This suggests that such emulsifiers can be a valuable nutritional strategy to improve resilience in aquaculture systems facing rinsing temperatures and heat stress, maintaining production to reasonable levels.

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# IMPLEMENTING THE STRATEGIC GUIDELINES FOR EU AQUACULTURE: EUROPEAN COMMISSION STAFF WORKING DOCUMENTS ON PLANNING OF SPACE AND ACCESS TO WATER FOR MARINE AQUACULTURE AND ACCESS TO SPACE AND WATER FOR FRESHWATER AND LAND-BASED AQUACULTURE

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The Commission Staff Working Documents (SWDs), on "Planning of space and access to water for marine aquaculture"[1] and "Access to space and water for freshwater and land-based Aquaculture"[2], serve as complementary resources aimed at implementing the "Strategic Guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030"[3]. Together, the two SWDs, provide comprehensive guidance for authorities and policymakers in Member States (MSs), addressing the allocation of space and access to water for both marine and freshwater aquaculture activities.

The SWDs were produced by the European Commission in collaboration with the Aquaculture Assistance Mechanism[4] (AAM) employing a thorough methodology to select good practices from EU and non-EU countries on the topic access to space and water. The methodology includes desk research and tools, such as: surveys, technical seminars, a workshop held in Brussels in 2023, and several online interviews with European aquaculture experts. Recently, e-learning modules were produced by the AAM on both documents to boost the good practices exchange among MSs.

The SWD on "Planning of space and access to water for marine aquaculture" explains social, economic, environmental, and regulatory conditions of the EU marine aquaculture sector, and identifies key challenges faced by public authorities with competencies in marine aquaculture spatial planning.

Similarly, the SWD on "Access to space and water for freshwater and land-based aquaculture", addresses: lack of social acceptance, conflicts with other activities, cost of space, water rights and licenses, application of the Water Frame Directive (WFD) and River Basine Management Plans (RBMPs) which, among others challenges, are factors that are restraining the competitiveness and further development of the freshwater and land-based aquaculture sector in the EU.

Both SWDs outline the necessary steps for effective access to space and water for all types of aquaculture and consolidate existing good practices and tools. The factsheets included in the SWDs address solutions to the identified challenges and provide practical example from both EU and non-EU countries. The factsheets, which emphasise the benefits of the selected good practices, also provide guidance to EU public authorities on how to implement such solutions, to enhance the planning processes, and ensuring sustainable aquaculture development in the European Union.

1. https://aquaculture.ec.europa.eu/key-documents/access-space
2. https://aquaculture.ec.europa.eu/key-documents/access-space-and-water-freshwater-and-land-based-aquaculture
3. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52021DC0236
4. The AAM is part of a Service Contract jointly managed by the European Climate, Infrastructure and Environment Executive Agency (CINEA) and DG MARE. The consortium is coordinated by NTT DATA, Poseidon, SCOPE - Netcompany, the European Aquaculture Society (EAS) and the European Aquaculture Technology and Innovation Platform (EATIP). https://aquaculture.ec.europa.eu/

# PRELIMINARY DATA ON STRESS BIOMARKERS IN *Anemonia sulcata*

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## Introduction

The sea anemone *Anemonia sulcata* is considered a highly valuable species in Andalusia (Spain) and, consequently, its wild stocks are significantly decreasing due to overfishing. The culture of this species could contribute to mitigate the fishing pressure and promote the restoration and long-term conservation of *Anemonia sulcata* populations. Nevertheless, there is a lack of information about its physiology and behaviour, necessary for optimizing culture conditions.

To enhance the health, growth, welfare, and productivity of cultured species, it is essential to identify and minimize potential stressors. Understanding the physiological status of the animals is crucial for determining the presence and severity of stress. This assessment is commonly performed through the analysis of stress-related biomarkers, including metabolites linked to physiological responses (e.g., lactate, glucose, cortisol) and the activity of antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase).

The aim of this study was to assess whether specific stress-related metabolites and antioxidant enzyme activities could be detected in *Anemonia sulcata* following exposure to various acute and chronic stress conditions.

## Materials and methods

Anemones were collected from 3-4 m depth at La Herradura beach (Granada), and afterwards acclimated in highly-aerated seawater tanks at IFAPA Centro "Agua del Pino" (Cartaya, Huelva). For this study 39 anemones (mean wet weight 25.11 ± 3.57 g) were randomly placed into 9 circular tanks exposed to natural temperature and light cycles. They were fed three times a week with commercial fish feed (pellets). The experiment was started after an acclimation week. 12 anemones were assigned to each of the three treatments: control (CTRL), normal culture conditions; Acute stress (A), animals exposed for six hours to a desiccation period outside the cultivation tank; **Chronic stress (C),** animals kept in darkness and starvation for 10 days. Each treatment was performed in triplicate. The experiment period lasted 45 days. At the end of the trial, pedal tissue samples were collected to analyze stress-related metabolites (lactate and glucose) and the activity of antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD).

## Results and Discussion

An independent samples Student's *t*-test was conducted to detect significant differences between treatments for glucose and lactate levels and GPx and SOD activity. Statistically significant differences were detected for all biomarkers (Table 1).

**Table 1.** Glucose and lactate concentrations, and glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities in control (CTRL) anemones and those exposed to acute stress (A) and chronic stress (C). Mean ± SE values for every biomarker and treatment. Asterisks indicate significant differences regards the control group.

| | TREATMENTS | | |
|---|---|---|---|
| BIOMARKER | CTRL | A | C |
| GLUCOSE (mg/g) | 0.21±0.12 | 0.70±0.23 * | 0.50±0.14 * |
| LACTATE (mg/g) | 0.09±0.03 | 0.19±0.05 * | 0.11±0.05 |
| GPx (U/g of protein) | 0.18±0.06 | 0.23±0.03 * | 0.12±0.06 |
| SOD (U/g of protein) | 7.30±4.88 | 1.64±2.24 * | 1.00±0.70 * |

*(Continued on next page)*

These findings indicate that stress in sea anemones induced a metabolic and antioxidant response. Elevated glucose levels were observed in both acute and chronic stress conditions, likely to facilitate rapid mobilization of energy reserves during stress exposure. Regarding lactate, anaerobic metabolism was detected under acute stress, characteristic of hypoxic conditions; however, no significant differences were found during prolonged stress, suggesting an adaptive adjustment of basal metabolism by the anemones over time. In terms of the antioxidant response, GPx activity was upregulated in response to increased reactive oxygen species (ROS) during acute stress, whereas chronic stress led to a decrease in GPx activity, although this reduction did not reach statistical significance, potentially indicating depletion of antioxidant reserves. Lastly, the marked reduction in SOD activity observed under both acute and chronic stress suggests a compromised antioxidant defense system, with the effect being particularly pronounced under chronic stress conditions.

**Conclusion**

The stress-related biomarkers lactate, glucose, GPx activity, and SOD activity were detected in *Anemonia sulcata* following exposure to stress conditions, suggesting their suitability as reliable indicators of welfare status in the culture of this species. Further studies will be necessary to test other stress markers commonly used in aquaculture.

**Acknowledgements**

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# FISH OF HOPE: AQUACULTURE WITH LITTLE WARRIORS IN THE HOSPITAL

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## Introduction

Pediatric cancer is the leading cause of childhood death in Spain. The severity of this disease causes Post-Traumatic Stress Disorder (PTSD) mainly in parents. Previous studies (López, 2019) agree on the importance of generating positive stimuli that produce Post-Traumatic Growth (PTG). This is where the European Project IGNITION acts as a link by studying the improvement of welfare and health in aquaculture. In this context, the training action will show children, during and after a PTSD process, the world of aquaculture, giving the opportunity to learn with their families its functioning to extrapolate it to their own medical circumstances. This will have special emphasis on the interrelationship of fish and healthy eating with human and environmental welfare ("One Health concept"), generating a positive action that directly brings science closer to children and their families. Through the use of different game dynamics, they will discover the similarity between the medical tests performed on them during their treatments and the tests we conduct to assess the health status of our farmed fish. By using positive actions like these, childrens were able to forget where they were and lose some of their fear of certain tests they undergo, such as a simple blood draw.

## Materials and methods

The activities were carried out at Hospital Puerta del Mar in Cádiz and Hospital Universitario de Jerez, once a week each for a period of 2 months. The target audience was children aged 4-14 years who were hospitalized for various pathologies or who attended the hospital for oncological treatment. The total number of people who participated in the workshops was around 100, including children and their families. Throughout the period in which the workshops were held, various activities were implemented with the aim of bringing scientific knowledge of aquaculture closer to hospitalized children and their families with a playful approach adapted to the different ages of the attending public. All activities had a dynamic, playful, and easy-to-understand basis for the target audience, even creating didactic prototypes to bring and introduce the aquaculture world in such a different environment as a hospital.

During the workshops, 4 types of activities were carried out:

1. "Unsustainable Jenga" game to demonstrate the fragility of marine ecosystems.
2. Experiment on varied and healthy eating (fish as part of it) vs. processed foods.
3. Visualization of aquaculture species used for human feeding.
4. Scientists for a day: simulation of an aquaculture sampling.



**Figure 1**. Images during and after the completion of the workshop in the playroom of the Hospital Puerta del Mar de Cádiz and Hospital Universitario de Jerez. Own elaboration.

*(Continued on next page)*

At the end of the workshop, the children became little researchers for a day, learning about our work as researchers and how our work is focused on obtaining more sustainable diets in aquaculture, ensuring and guaranteeing animal welfare. In order to bring the aquaculture world closer in a realistic way, and given the impossibility of handling live animals in a hospital environment, an alternative was sought through the use of stuffed fish, allowing children to identify and touch aquaculture species, and draw blood from them in the same way researchers done to check animal welfare. The development of a didactic prototype for blood extraction simulation and video projection reinforced the set of activities, connecting them with scientific reality, which was highly valued by the adult audience.

**Results and discussion**

These sessions allowed participants to explore the complex aquatic ecosystem, understand the importance of sustainability in marine resource management, and the importance of aquaculture and science in all of this, with aquaculture being the central axis of all activities as a sustainable alternative for marine protein production in the growing global food supply. Although the hospitalized children presented various pathologies and moods, both the type of activities and the topic addressed managed to connect with each of them and enthuse them, making their participation very active. With the general objective of bringing science closer to children undergoing oncological treatment and their families, and promoting the consumption of aquaculture fish as a healthy and sustainable option through outreach activities and simple experiments, children learned to distinguish healthy foods from processed products, while adults discovered the nutritional and environmental benefits of aquaculture as a safe and balanced food source.

During the period the workshop lasted, both children and adults became researchers, transforming hospital playrooms into small laboratories, an activity always focused on seeing the similarity with medicine and their hospital status.

**Conclusions**

Our work demonstrates a different approach to aquaculture outreach, bringing this world to an unusual environment. These activities not only allow participants to disconnect from their surroundings but also discover a world unknown to most of them, such as aquaculture. Through communication mainly with the adult audience, we discovered that it remains a little-known sector, which shows the need to continue and emphasize closer and more effective communication. Likewise, the healthcare staff showed their interest in carrying out such uncommon workshops in the hospital playrooms (while waiting for oncological treatments) for understanding the significance healthy eating with the consumption of fish in the diet. These areas are a good place for learning as long as the objective of these rooms is not lost. Thanks to the feedback we received from the participants, it has been one of the most enriching experiences for all those who participated in this project, as conveyed by the families involved.

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# AQUACUL… WHAT?
# THE IMPORTANCE OF BRINGING AQUACULTURE SECTOR CLOSE TO A NON-STRICTLY SCIENTIFIC AUDIENCE

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## Introduction

In recent decades, Spain has become one of the main aquaculture producers in Europe. However, aquaculture remains largely unknown and undervalued by part of society, reflecting a limited understanding of the scientific research aimed at promoting its sustainable development. This disconnect also influences consumer perception and acceptance of aquaculture products, which are sometimes viewed with skepticism despite their high quality and environmental benefits. Moreover, despite the Spanish education law (LOMLOE 3/2020) integrating the Sustainable Development Goals (SDGs), sustainable aquaculture, intrinsically linked to several SDGs (1, 2, 8, 12, 14), is not a topic considered for working on the SDGs. Working on the SDGs from an early age becomes important to achieve this issue (Cernev and Fenner, 2020). It is therefore crucial to disseminate the advances of this sector beyond the scientific realm to mitigate the social stigmatization derived from a lack of understanding. Bringing these concepts closer to future generations is fundamental for effective knowledge transfer that fosters a positive connection between science, aquaculture, and society, aligning actions within the 2030 Agenda: 'Think globally, act locally to achieve change'.

## Materials and methods

This study was conducted with the aim of bringing the aquaculture world closer to primary students. Through an active methodology, activities and audiovisual materials, it highlighted the value of farmed fish and its research thanks to the correlation between: i) fish as an important source of omega 3, ii) the importance of stablishing good nutrition in cultivated fish larvae with sustainable raw materials, and iii) the respect for the marine environment. These activities were carried out in two Primary Schools in Spain, one located in the Bay of Cádiz with a culture close to the sea (C.I.E.P Arquitecto Leoz), and the other located in Madrid, without such marine connection (Colegio Aquila). The workshops were carried out over two consecutive days and involved a total of 433 students, aged between 9 and 13 years. Before starting the activities, a concept test composed by 4 simple questions was administered to all students using a game called "Port and starboard" to be answered with either "Yes" or "No":

***Q1:*** Do you like to eat fish?
***Q2:*** Is the origin of all fish that we consume from extractive fishing?
***Q3:*** Do you know what overfishing is?
***Q4:*** Do you know what aquaculture is?

After the initial test, and considering its results, various practical and visual activities were carried out. These interventions were addressed to introducing concepts and knowledge that the students lacked, in order to gain a significant understanding of aquaculture and scientists' advances, making it more sustainable.

## Results and discussion

From the 433 primary students, 55.4% (n=240, within both locations) were from the 4$^{th}$-grade, 22.2% (n=96) from 5$^{th}$-grade, and 22.4% (n=97) from 6$^{th}$-grade. After analyzing the results, 75.5% of the students like to eat fish, and 81.1% considered that all the fish they consume come from extractive fishing. However, 67.4% did not know the meaning of overfishing, and 94% did not know what aquaculture is. These results also remark a clear age-dependent factor mainly related to both Q2 and Q3 questions, but not in the case of Q4.

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Likewise, within the totality of 4th-grade primary students, two groups were differentiated according to their living area, either near the coast (Cádiz, 40.4%) or inland on the Iberian Peninsula (Madrid, 59.6%). Focusing on these sub-groups, the results showed that, regarding question Q1, 81.4% of coastal students like to eat fish, compared to 72% of inland students. Moreover, answers to Q2 show that 94.8% (Cádiz) and 96.5% (Madrid) of students consider that fish they consume come from extractive fishing. Besides, only 13.4% of coastal students knew the term overfishing (Q3) and 3.1% the term aquaculture (Q4) compared to 7% and 2.8%, respectively, of the students living inland on the Iberian Peninsula.

These results show that most primary students believe that the fish they consume comes from extractive fishing and, therefore, the term of aquaculture is completely unknown to them. This hiaghlights that 4th grade of primary school is the educational moment when students begin to work more autonomously (Casado Berrocal and Castro Bayón, 2017), being an ideal stage to acquire the knowledge and habits that allow them to use the environment sustainably, becoming future agents of change (Ruiz et al., 2025).

Implementing the concepts of sustainable aquaculture within educational programs through gamification and collaborative learning as a methodological approach will enable students to receive an education in future consumption from an ecological and sustainable perspective (Torralba Burrial and Dopico Rodríguez, 2021). Furthermore, the introduction of innovative and playful activities in education, as has been the case, is a recognized phenomenon to support the learning process (Makri et al., 2021).

**Conclusions**

The presentation of these results through the use of creating specific activities for this purpose generates a positive connection between science, aquaculture and society, cultivating interest from an early age. It is important that these messages with the audience do it, although a "bottom-up" knowledge transfer design is needed to ensure complete assimilation at more complex levels. Despite Spain's deep relationship with the sea and its products, educational curricula tend to prioritize terrestrial agriculture, revealing a notable absence in the teaching of aquatic cultivation.

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# MICROBIOME PROFILING OF SUBMARINE WASTEWATER OUTFALLS FOR COASTAL WATER QUALITY MONITORING IN THE GULF OF VALENCIA

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## Introduction

The Valencia metropolitan area, positioned along the Gulf of Valencia, confronts major pollution issues from urban and industrial discharges. Effective monitoring of water quality is critical, but traditional methods relying on physical-chemical and microbiological analyses are time-consuming and require skilled personnel, making them impractical for large-scale monitoring. In this preliminary study, we perform a year-round microbiome composition assessment of the water column from several discharge points from selected submarine outfalls linked to wastewater treatment plants, with the aim of detecting seasonal biomarkers for an enhanced monitoring of coastal water quality.

## Methodology

Samples were collected in three replicates from four distinct submarine outfall discharge points and two farther points controls, across all four seasons of the year. Water sampling (5L) was conducted aboard the IMEDMAR-UCV research vessel using a peristaltic pump, collected at 1 meter depth and filtered through 0.45 μm MCE sterile filters. DNA was extracted using a proprietary protocol (unpublished). DNA libraries were constructed through the amplification of prokaryotic 16S rDNA (Caporaso et al. 2012) and sequenced using the Illumina® technology. DNA sequences were analyzed with MOTHUR and statistical analyses were performed using RStudio and tools from different packages such as DESeq2, Microeco, Vegan and FAPROTAX.

## Results

The comparative analysis performed with *Linear Models for Differential Abundance (LinDA)* (Figure 1A) showed significant differences in the relative abundance of several bacterial taxa between the control site and the submarine outfall points. The latter showed a marked increase in *Sulfurimonas*, *Fusibacter*, *Candidatus Peribacteria*, *B2M28*, *Candidatus Komeilibacteria*, Peptostreptococcales–Tissierellales, and *Desulfosarcinaceae*, all with statistically significant differences ($p < 0.05$). In contrast, other groups such as *Bdellovibrio* and *Pir4 lineage* showed no relevant changes between conditions. Functional inference of metabolic functions using FAPROTAX (Figure 1B) showed that most processes displayed similar relative abundances in outfall and control sites, except for pathways related to the respiration of sulfate and sulfur compounds, which were significantly higher in the outfall samples, whereas the control sites exhibited comparatively higher values for other oxidation and methylotrophy pathways.

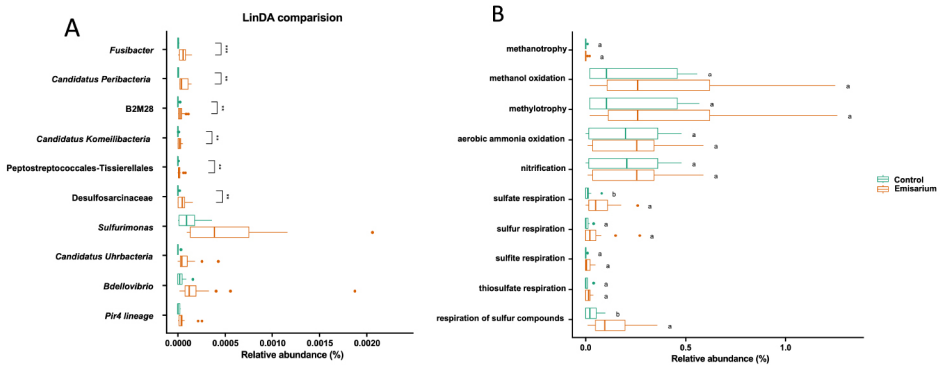


Figure 1. (A) Differential abundance analysis performed with LinDA comparing microbial communities between the control and outfall (*Emisarium*) sites. (B) Functional profiling of metabolic pathways showing significant differences between sites based on FAPROTAX annotation.

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**Discussion**

Our data revealed significant shifts in the microbial community composition between the control and submarine outfall sites, indicative of environmental impacts associated with the outfall discharges. The notable enrichment of taxa such as *Sulfurimonas* or *Desulfosarcinaceae*, among others, in the outfall samples suggests a selection for microorganisms adapted to sulfur-rich or reducing conditions, such as seen in other studies linked to polluted marine environments (Zhang et al., 2007; Chen et al., 2019). Furthermore, functional inference underscored these taxonomic shifts, confirming a biogeochemical alteration toward a sulfate and sulfur-driven microbial ecosystem, possibly derived from a more anaerobic and sulfur-rich environments on these sites.

**Conclusions**

Overall, these results highlight the influence of wastewater submarine outfall discharges on microbial community structure and function, favoring sulfur-metabolizing bacteria and altering biogeochemical cycles.

**Acknowledgements**

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# ASSESSING THE IMPACT OF CLIMATE CHANGE - DRIVEN ACIDIFICATION IN THE ADRIATIC SEA ON BYSSUS PRODUCTION, BEHAVIOUR, AND SHELL BIOMINERALIZATION IN *Mytilus galloprovincialis*

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**Introduction**
The increase in atmospheric $CO_2$ levels is causing a progressive acidification of sea water. Projections indicate a decrease in ocean surface pH by approximately 0.3–0.5 units by the year 2100. This future condition could affect calcifying marine organisms, particularly bivalves, due to their reliance on carbonate ions for shell formation. *Mytilus galloprovincialis*, the Mediterranean mussel, is both ecologically important and economically valuable in European aquaculture. This study aims to investigate how predicted acidification scenarios may influence mussel byssus production, gregarious behaviour, shell integrity, and molecular responses related to calcification.

**Materials and Methods**
*M. galloprovincialis* (n = 288; shell length 3.5 ± 0.5 mm) were collected from a commercial mussel farm in the North Adriatic Sea and acclimatized in controlled laboratory aquaria. The mussels were then randomly assigned to four experimental pH treatments (8.2 – control, 7.9, 7.7, and 7.4), simulating current and predicted future ocean conditions based on IPCC projections. pH was modulated and stabilized using $CO_2$ bubbling systems over a 90-day exposure period. Total alkalinity was measured weekly to determine carbonate system parameters, including aragonite and calcite saturation state.

Byssus thread production was assessed at multiple intervals (1, 3, 6, 9, 12, 18, and 24 hours). Mussel positions were recorded on a grid at the bottom of aquaria, to quantify clumping events in aggregation behaviour and crawling speed (mm/h).

Shell quality was evaluated through scanning electron microscopy (SEM), mechanical strength testing under pressure, and mineralogical analysis via X-ray diffraction.

Histological analyses of mantle tissue were conducted using Alizarin Red S staining to localize calcium deposits useful for shell formation.

Furthermore, expression levels of key genes associated with biomineralization (chitin synthase, calponin-like protein, carbonic anhydrase, tyrosinase 1–2, chinase, plasma membrane $Ca^{2+}$ ATPase and sarcoplasmatic $Ca^{2+}$-binding protein) were quantified using RT-qPCR on outer and inner mantle tissue samples.

**Results and Discussion**
No significant decrease in byssus production was observed between pH treatments, only an increase over time (Fig.1). But behavioural alterations emerged such as decreased clumping (Fig.2) and mobility under low pH conditions. Mussels exposed to acidified seawater compromised shell ultrastructure and mechanical properties, with altered mineral phase composition. At the tissue level, a redistribution of calcium within the mantle parts was observed, suggesting disruptions in shell formation processes. Gene expression analyses revealed downregulation of biomineralization-related genes under low pH, indicating impaired physiological regulation of shell formation.

**Conclusion**
This study provides a comprehensive assessment of the effects of ocean acidification on *M. galloprovincialis*, integrating behavioural, morphological, and molecular endpoints, it highlights the species' sensitivity to low seawater pH, with implications for mussel productivity under future climate scenarios.

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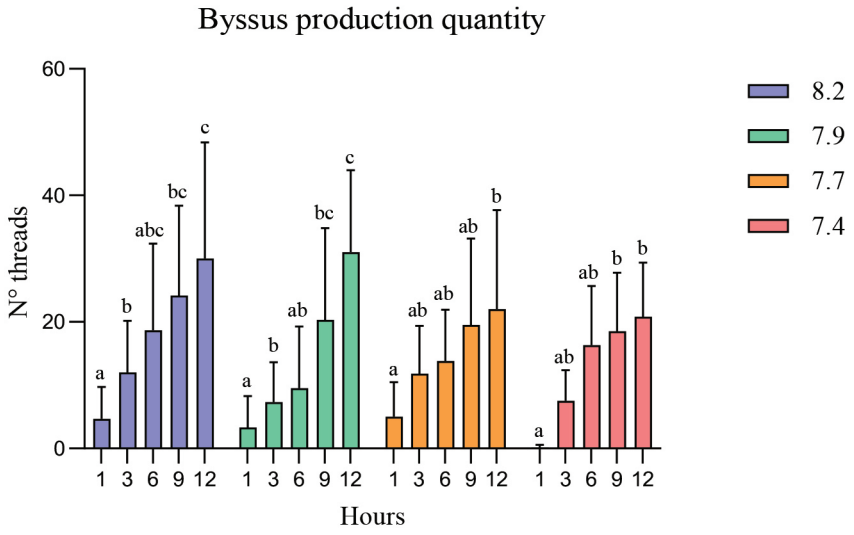


Fig. 1: Byssus production quantity in the four pH treatments at different hours of acidification exposure. The lowercase letters (a,b,c) represent significant differences in Two-way ANOVA ($P < 0.05$) between times for the same pH treatment.

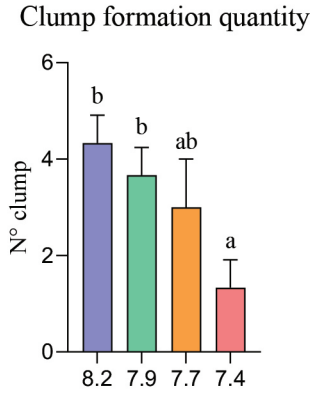


Fig. 2: Clump formation quantity in the four pH treatments (in the first 24 hours of exposure). The lowercase letters (a,b) represent significant differences in One-way ANOVA ($P < 0.05$) between pH treatments.

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# MICROBIOTA OF *Manila clam* SPAT PRE-FATTENED IN DIFFERENT FARMING TECHNOLOGIES

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**Introduction**
Microbiota plays a critical role in bivalve health and resilience. Farming systems for Manila clam could optimize growth and influence the associated microbiota, which also depends on environmental conditions. This study aimed to compare three pre-fattening supports by evaluating the growth performance and composition of the clam microbial communities.

**Materials and Methods**
Three different pre-fattening systems were tested at NaturEdulis (Goro, Italy) for four months: net lanterns suspended in superficial sea water; poches in less superficial water, land-based upwelling tanks within the hatchery. Spat (4.68±0.53 mm in length) were monitored monthly. Six clams per group were sacrificed to measure growth parameters and to characterize the microbiota (alpha and beta diversity, microbiota composition at phylum level). At monthly sampling, microbial DNA was extracted using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany), and the V3–V4 hypervariable region of the 16S rRNA gene was amplified via PCR for high-throughput sequencing.

**Results and Discussion**
Clam growth was evident in all systems, with significant increases in length and total weight over time, particularly in the lantern support. This trend followed the Chl a concentration, which was generally higher in the lantern environment.

The microbial community composition of clams varied significantly across sampling times and support types. At T0, *Pseudomonadota* dominated, while *Bacillota* became more prevalent in later samples, with *Cyanobacteriota* dominating from June. In the final sampling, *Spirochaetota* and *Chloroflexota* were predominant in the poche support. Diversity increased over time, with T0 showing the lowest diversity.

**Conclusion**
The lantern support was identified as the most effective pre-fattening system, promoting superior growth performance. Microbiota shifts suggest that environmental factors and support type play a key role in shaping microbial communities. The poche system appeared to be the least efficient, likely due to mesh fouling, which reduced both water flow and microbial diversity.

**Funding**
This research was undertaken under the BIVALVI (ERA-NET BlueBio: Advancing European bivalve production systems) project, which has received funding from the European Union's Horizon2020 Research and Innovation programme under grant agreement no. 817992 (https://bluebioeconomy.eu/advancing-european-bivalve-production-systems/)

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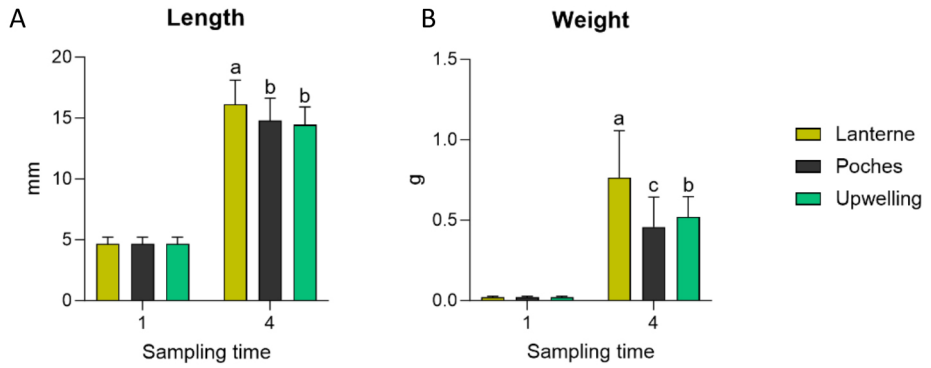


Fig. 1: Clam length (A), weight (B) at the beginning and end of the test. The lower letters (a,b,c) represent significant differences in two-way ANOVA ($P < 0.05$).

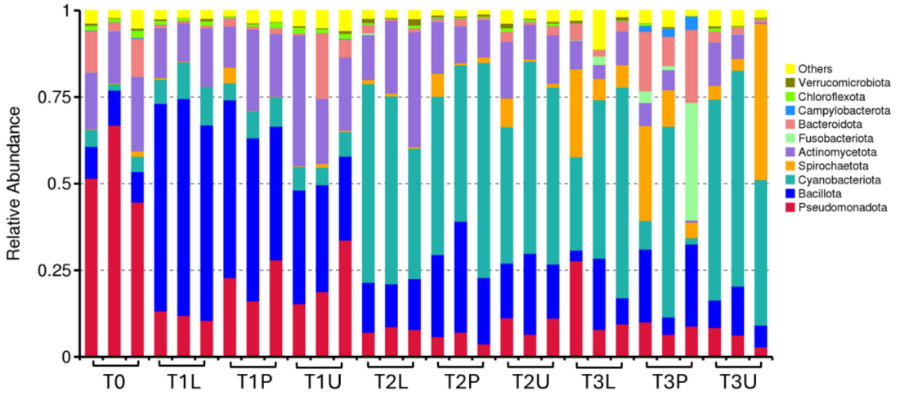


Fig. 2: Microbiota compositions of the clams in the different supports and sampling time (T0 = initial sampling in April, T1 = May sampling, T2 = June, T3 = July, L = lantern support, P = poche, U = upwelling tanks). Bar plot summarizing composition at phylum level.

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# COMPARISON OF TWO DIFFERENT METHODS (FRAP AND TEAC) TO EVALUATE THE TOTAL ANTIOXIDANT CAPACITY IN THE SKIN MUCUS OF RAINBOW TROUT (*oncorhynchus mykiss*) IN RESPONSE TO ACUTE HYPOXIC STRESS

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**Introduction**

Skin mucus is an essential protective barrier for fish, whose production increases in response to environmental stressors and pathogens (Subramanian et al., 2007; Vatsos et al., 2010). The total antioxidant capacity (TAC) of skin mucus is a valuable, non-invasive biomarker for assessing the welfare of fish and understanding its physiological response to environmental challenges. Previous studies (de Mercado et al., 2018) showed that TAC, measured by the Ferric Reduction Activity Power (FRAP), changes in response to stress. However, TAC can be assessed by different methods that are sensitive to different groups of antioxidants. FRAP is based on the reduction of ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$) by antioxidants through a SET (Single-Electron Transfer) mechanism, making it especially sensitive to water-soluble compounds such as vitamin C, polyphenols and glutathione. In contrast, the TEAC (Trolox Equivalent Antioxidant Capacity) assay determines the capacity to neutralize the ABTS radical, both by the SET mechanism and by the HAT (Hydrogen Atom Transfer) mechanism, which allows it to detect a wider range of antioxidants. Given these differences, the study's objective was to compare the kinetics of the TAC response in rainbow trout skin mucus after acute hypoxia stress, using both the FRAP and TEAC assays to see if they provide different insights into the antioxidant response.

**Material and Methods**

Thirty rainbow trout (76.3 ± 2.6 g; length 17.9 ± 0.1 cm) were subjected to acute hypoxia stress, and skin mucus were collected at 4 time points, with six fish sampled per point: pre- stress (basal), immediately post-stress (time 0), and at 2- and 5-hours post-stress. The samples were collected and processed according to Mercado et al. (2018). FRAP analysis was performed using the method described by Benzie and Strain (1996), where reducing capacity was determined by measuring absorbance at 593 nm against a $Fe^{2+}$ standard curve. Results were expressed in µmol $Fe^{2+}$/g of skin mucus. The TEAC assay was analysed following the instructions of the commercial OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC) Assay Kit (ABTS), and the absorbance was measured at 405 nm against a Trolox standard curve. Results were expressed in µmol Trolox/g mucus. Differences between time points were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

**Results and discussion**

The different methods used showed a different response. The FRAP assay showed no immediate change in TAC after acute hypoxia stress (0H). However, a significant increase was observed at 2H post-stress respect to basal level ($P<0.05$), which subsequently decreased to intermediate levels at 5 hours (Fig 1). This delayed response suggests that the antioxidant response is not immediate. Conversely, the TEAC method detected no significant changes in TAC at any of the sampling times (basal, 0H, 2H, 5H) but showed similar values to those observed in with this technique by other authors (Guardiola et al., 2018; Tagami and Kuwahara 2020). Although a slight increase was observed at 0H, this was not statistically significant ($P>0.05$).

These results suggest that the antioxidant response to hypoxia in skin mucus can be multifaceted. The choice of assay is critical. FRAP could be more sensitive to water-soluble antioxidants and low molecular weight compounds that are mobilized or synthesized as a late response to hypoxic stress, while TEAC could detect more structural antioxidant compounds that are not easily or quickly modified. This highlights the need for further research to fully understand the complexities of this biological defence mechanism.

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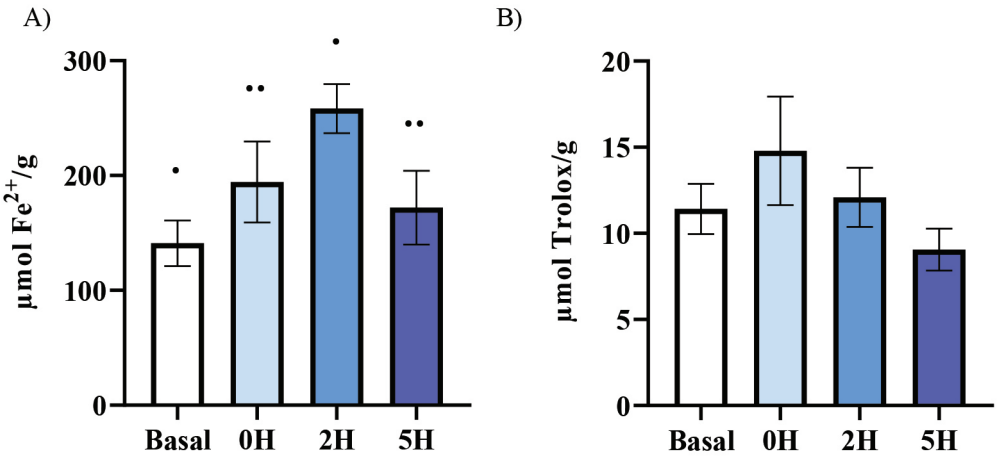


Fig 1. Evaluation of the total antioxidant capacity (TAC) in the skin mucus of rainbow trout (*Oncorhynchus mykiss*) at different time points: pre-stress (basal), 0H (immediately post-stress) and 2H and 5H post-stress. (A) FRAP values (µmol $Fe^{2+}$/g mucus). (B) TEAC values (µmol Trolox/g mucus). Data are presented as mean ± standard error (SEM). [a, b]Indicates statistically significant differences ($p < 0.05$).

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# ENVIRONMENTAL ASSESSMENT OF RAINBOW TROUT PRODUCTION IN FRANCE: A CASE STUDY IN HAUTS-DE-FRANCE REGION

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## Introduction

With the ongoing growth of aquaculture, there is an increasing societal concern around reducing environmental impacts of production systems, particularly for rainbow trout (*Oncorhynchus mykiss*) the main fish species farmed in France (33082 tonnes; FAO, 2023). Addressing these concerns requires robust and standardized methods to assess such impacts.

Life Cycle Assessment (LCA) offers a standardized and comprehensive framework to evaluate environmental impacts of fish production systems by analysing material and energy flow across multiple impact categories. This method was extensively applied in previous studies on salmonids production (e.g., Chen et al, 2015; Pouil et al., 2024).

In this study, we applied LCA to assess the environmental impacts of rainbow trout farming in the Hauts-de-France region, currently the leading production area in France, with its share increasing from 21.9% in 2016 to 28.3 % in 2023 of national volumes (Agreste, 2019; Agreste, 2025) while this northern region may become increasing strategic for trout production in the climate change context as it is less affected by water scarcity.

## Materials and Methods

Between March and May 2025, eight rainbow trout farms using conventional flow-through systems in Hauts-de-France were surveyed. The data collected supported an attributional LCA following ILCD guidelines (JRC, 2010), with a cradle-to-farm-gate scope and a functional unit of one tonne of trout produced per year. Primary data came from on-site surveys; background data were sourced from Agribalyse 3.0 and Ecoinvent 3.3.1. The Life Cycle Inventory covered five subsystems: feed production, fry production, farm operations (i.e. use of facilities and equipments and emissions of pollutants from the biological transformation of fish feed), land emissions, energy use, and infrastructure. Environmental impacts were assessed across six categories: global warming (GWP), terrestrial acidification (TAP), freshwater eutrophication (FEP), marine eutrophication (MEP), land use (LU), water dependence (WD) using ReCiPe 2016 Midpoint (H) v1.07, and Cumulative Energy Demand (CED). To enable comparison with previous studies, the environmental impacts per tonne of trout produced were also assessed using the CML baseline methodology. The results were analysed to identify key contributors and compare the performance of the farms at a regional scale.

## Results

We observed marked differences in environmental performance per tonne of farmed trout between production sites, particularly in the TAP, FEP, and LU categories, where impacts varied by up to 2.5-fold (1.9 to 2.5 times) between the lowest (Site 2) and highest-impact (Site 6) sites. Across all sites, feed was the main contributor to environmental impact, accounting, on average, for GWP, 87% of TAP, 95% of LU, and 67% of CED. Farm operations were the main drivers of FEP (89%) and MEP (84%), as well as WD (85%). Other subsystems contributed only marginally.

The most notable change in the contributions between sites was linked to fish feed consumption and the intensity of liquid oxygen use responsible for 56 to 87 %, respectively 0.6 to 31% of GWP value. Electricity consumption (1.9-5.4%) and fry production (1-6.2%) also showed minor differences across sites.

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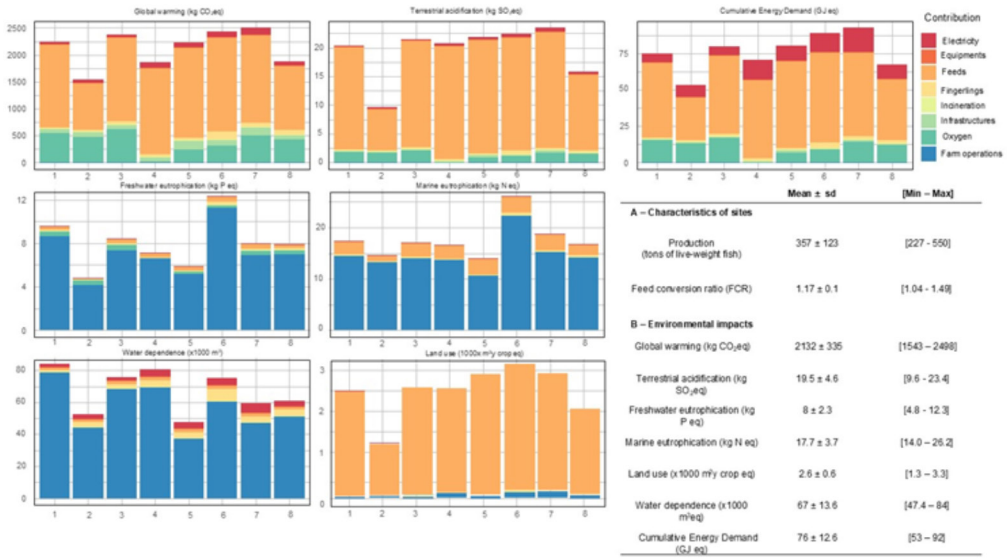


**Fig. 1.** Contribution of rearing system components to the environmental impacts (assessed using ReCiPe 2016 Midpoint (H) v1.07 methodology) of eight trout farms in Hauts-de-France. Results are expressed per tonne of live-weight trout at the farm gate. The figure includes site characteristics and values for the seven selected impact categories (mean ± standard deviation, min-max).

## Discussion

Environmental impacts assessed using the CML baseline methodology for the eight trout farms in Hauts-de-France were consistent with values obtained with the ReCiPe method and with values reported for conventional flow-through systems (reviewed by Pouil et al., 2024). A comparison with Chen et al. (2015), who surveyed 10 French farms producing between 100 and 500 tonnes annually, revealed higher regional averages in Hauts-de-France for TAP and CED (1.5 and 1.6 times higher, respectively), likely due to more energy-intensive equipment and operations. In contrast, water dependence was 1.7 times lower, possibly reflecting higher production intensities.

Feeds were among the main contributors to environmental impacts while variations in the contribution of feeds across sites were probably linked to differences in composition and transport distance. As expected, we found a strong correlation between FCR with impact levels across most categories.

This analysis forms part of a broader PhD project aiming to assess trout production impacts at the national scale. This work, carried out as part of the CoBreeding project and Metaprogramme Climae, received support from the French government managed by the National Research Agency under the France 2030 initiative, reference ANR-22-PEAE-0003.

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# PATHOGEN-SPECIFIC DYNAMICS IN SHRIMP DISEASE MODELS: A COMPARATIVE STUDY OF INDIVIDUAL AND GROUP CHALLENGES FOR WSSV AND AHPND IN *Penaeus vannamei*

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*Penaeus vannamei*, the Pacific whiteleg shrimp, is a cornerstone species in global shrimp farming due to its fast growth, adaptability, and high market demand. However, disease outbreaks, particularly those caused by White Spot Syndrome Virus (WSSV) and Acute Hepatopancreatic Necrosis Disease (AHPND), continue to represent major constraints to production (Brummett et al., 2014). While controlled and standardized experimental challenge models for these diseases are critical for evaluating prophylactic and therapeutic interventions, they are still scarce. For progressing on this topic, reliable detailed data on disease pathogenesis and dynamics is paramount. Comparative data on individual versus group challenge approaches - especially across different pathogens - can bring substantial added value to the process of the development and improvement of disease challenge models. However, these data remains virtually inexistent.

In this study, we established and compared both individual and group challenge models for WSSV and AHPND in *P. vannamei* under controlled laboratory conditions. Individual challenge models were designed to standardize pathogen exposure and assess the efficacy of health management products with high precision. Group challenge models, meanwhile, were developed to simulate more natural disease transmission dynamics and allow for broader application of candidate treatments.

For WSSV challenges, one peroral inoculation with WSSV-infected tissue inoculum (Thai-1 strain) (Thuong et al., 2016) was used in both individually housed (1 shrimp per 10L tank) and group housed challenges (100 shrimp per 290L tank and 10 shrimp per 10L). AHPND disease challenges were performed in the same setups but shrimp were inoculated via immersion with *Vibrio parahaemolyticus* TW01 (Alloul et al., 2021). Also in these cases, one single inoculation was performed. In both cases, animals were maintained for at least 10 days post inoculation and disease and mortality were monitored several times per day.

Striking differences were observed between pathogens and challenge models. During both the individual and group WSSV challenges, the Thai-1 strain had an incubation period of 24-54 hours post infection (hpi) and an irreversible disease progression within 78 hpi. In WSSV group challenges, a distinct pattern of reinoculation events was demonstrated, resulting in 100% final mortality in all cases (following a minimal stocking density). This progression differed markedly from the individual WSSV challenge, where mortality curves were less steep and final mortality much more limited, suggesting differences in infection dynamics and host response depending on the mode of exposure.

In contrast, AHPND challenges showed comparable disease dynamics between individual and group models. The *V. parahaemolyticus* TW01 strain resulted in mortality within 12 hours following inoculation, with an exponential mortality increase in the first 36 hours. Notably, AHPND did not result in irreversible disease progression in the early stages post-challenge. Mortality was variable depending on the trial and dosing, but final cumulative mortality stabilized between 50% and 85%. In addition, higher initial bacterial loads were required to establish consistent infections in AHPND group models in comparison with individual challenges, highlighting differences in infectious dose thresholds. This was not observed in WSSV challenge models at our facility. These findings underscore the importance of understanding pathogen-specific dynamics under different circumstances, when designing and interpreting challenge studies.

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This work emphasizes that while individual challenge models offer high reproducibility and control, group challenges better reflect field-like transmission and reinfection dynamics, especially in the context of WSSV. For AHPND, the choice of model may be less critical given the similar progression observed across methods, but attention to initial dosing remains essential. This strongly suggests that the transmission of WSSV between individuals plays a very important role in field outbreaks, while this seems to be less important in AHPND, where water transmission independently from the host might be of greater importance.

In conclusion, the choice of challenge model has a significant impact on disease outcome and must be tailored to the specific pathogen and research objectives. This study contributes valuable insights for the development and refinement of disease challenge protocols in *P. vannamei*, ultimately supporting more effective evaluation of health management strategies in shrimp aquaculture.

This research received partial funding from Flanders Innovation and Entrepreneurship (Belgium).

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# SIMPLIFIED ROTIFER PRODUCTION: A KEY TO HIGH-QUALITY ROTIFER ENRICHMENT

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## Introduction

The production of high-quality live food remains a key success factor in many hatcheries for marine finfish species. The required rotifer quantities, needed to successfully produce fish larvae, have decreased in the last years, thanks to the development of high-quality, partial rotifer substitution diets such as Natura pRo (INVE Aquaculture, Thailand). Because of this trend, it is of uttermost importance that the provided rotifers are of high nutritional and microbial quality and that the procedures to produce them are easy and limited in time. In this work is presented how rotifer culture and enrichment combinations, using the correct products, can result in optimal live prey for the rearing of Seabream larvae.

## Materials and methods

*Rotifer culture and enrichment*

L-type rotifers were cultured in cylindroconical tanks of 850L at a temperature of 26°C and a salinity of 25ppt. The culture tanks were equipped with aeration in order to provide a homogeneous distribution of the rotifers and rotifer diets. Dissolved oxygen levels were kept between 80 and 150% of saturation. Rotifers were cultured using three different feed combinations, each designed to optimize the cultivation process:

1. 90% Fresh Baker's Yeast/10% RoBoost = (Control or CTR)
2. 100% VitAlgae Nanno (=Vitalgae Nanno)
3. 90% VitAlgae Nanno/10% RoBoost (=Vitalgae/Rob)

The required rotifers for Seabream larvae were enriched directly in the same culture medium, without a harvesting or rinsing step, at a density of 1000-1500 rotifers per ml. This enrichment was performed using Easy Dry Selco (INVE Aquaculture, Thailand), up to 2x145 ppm for a duration of 12 hours. At harvest, rotifers were rinsed and stored in cold storage (6-8°C) for the different feedings during the day.

*Seabream Larval rearing*

For each treatment, three replicate tanks of 1000L were stocked with 80 000 just-hatched Seabream larvae, all originating from the same pool of eggs. The larvae were reared at a temperature of 19±0.5°C in seawater with a salinity of 38ppt. From 3 to 20 days post hatch (dph), approximately 60-70g of VitAlgae Nanno per m³ were added daily to the tanks followed by a daily ratio of 15 grams of Sanolife GWS (INVE Aquaculture, Thailand) per m³ between 21 and 30dph. In all tanks, the larvae were fed following the traditional feeding protocol, using rotifers (produced according to the three different treatments) from 3 to 30dph, AF SEP-Art Artemia from 16 to 26dph and EG SEP-Art, enriched with Easy Dry Selco, from 23 to 55dph. At 19dph a dry diet (O.Range, INVE Aquaculture, Thailand) was introduced and fed until the end of weaning (55dph).

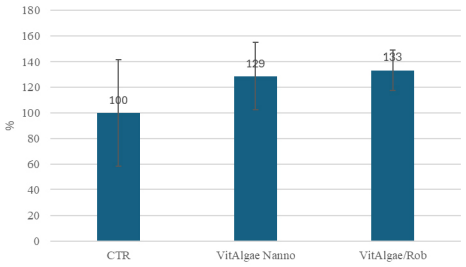


Fig.1. Relative rotifer production over a three-day batch culture period

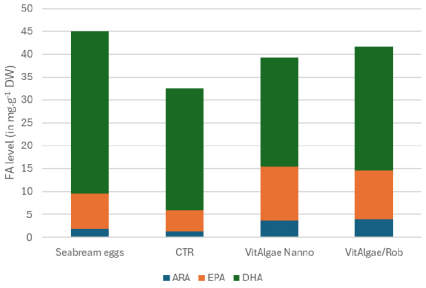


Fig.2. EPA, ARA, DHA Levels in Enriched Rotifers Compared to Seabream Eggs

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*Evaluation parameters*
The growth performance and nutritional profile of the rotifers were evaluated for all treatments.

For the Seabream larvae, survival rate, growth parameters, stress resistance, deformity levels and FAME values were assessed. Larvae were weaned at 55 dph and fish from all tanks were graded to determine the survival and quality of the fry produced.

**Results**
The average rotifer production over a three-day batch culture period, expressed in millions of rotifers reproduced per 1000 liters, was approximately 30% higher for treatments containing VitAlgae Nanno compared to the control treatment (see Fig.1).

FAME analyses for the essential fatty acids performed on the Seabream eggs and on the enriched rotifers from the three treatments, are presented in Fig.2. The sum of essential fatty acids (EPA, ARA and DHA) present in rotifers enriched with Easy Dry Selco directly in the rotifer culture medium after using VitAlgae Nanno either at 100% or as 90% (with 10% Rob), is similar to the levels found in Seabream eggs, and higher compared to the control rotifers. Regarding the performance of the Seabream larvae, all treatments yielded similar survival rates. The growth rate at the end of the rotifer phase was slightly higher for the treatments using VitAlgae Nanno, compared to the control treatment, as evidenced by both length and dry weight measurements. At 30 dph, fish larvae from treatments containing VitAlgae Nanno demonstrated better resistance when exposed to a salinity stress test than the control larvae, however, these differences were not apparent by the end of the weaning period. Histological staining analysis of early deformity levels at a total length of 10-12mm, did not reveal any differences between the treatments.

**Conclusions**
These results highlight the importance of high-quality rotifer culture and enrichment products in maintaining optimal larval development and performance, especially as the quantity of rotifers used in larval fish protocols decreases due to the introduction of new larval diets. As a conclusion it can be stated that the use of VitAlgae Nanno, RoBoost and Easy Dry Selco, applied during a combined culture and enrichment process for rotifers, results in a rotifer quality that is suitable for a successful larval rearing of Seabream larvae. These procedures, significantly reduce the manpower and resources, needed to produce high-quality rotifers without compromising the quality of the fry produced.

# MODULATION OF ARTEMIA MICROBIOTA THROUGH PROBIOTIC ENRICHMENT: A STRATEGY TO ENHANCE BIOSECURITY IN MARINE LARVICULTURE

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## Introduction

The increasing demand for fry is driving hatcheries to intensify production, often resulting in high rearing densities. Under such conditions, health management and microbiological control are crucial and essential factors for successful larval rearing. Among the many aspects to consider when defining a biosecurity plan, the management and control of live feed (primarily rotifers *Brachionus plicatilis* and brine shrimp *Artemia franciscana*) play a central role.

This study aimed to evaluate the effectiveness of a combined protocol based on the use of EG SEP-*Art*™ D-FENSE Artemia and a probiotic enrichment (Sanolife® MIC-F, INVE Aquaculture, Belgium) containing *Bacillus licheniformis, B.subtilis* and *B.pumilus,* to modulate the microbiota associated with *Artemia*, thereby contributing to improved larval health and biosecurity.

## Materials and Methods

Three enrichment strategies were tested:
**Control**: EG SEP-*Art*™ Artemia enriched without probiotic addition
**Probiotic Enrichment in Matured Water**: EG SEP-*Art*™ D-FENSE Artemia enriched in matured water with 1 $g.l^{-1}$ MIC-F (without prior activation) added 2 hours before nauplii introduction
**Post-Harvest Probiotic Treatment**: EG SEP-*Art*™ D-FENSE Artemia enriched under standard conditions, followed by treatment with activated MIC-F (2.5 $g.l^{-1}$, 5000 $nauplii.ml^{-1}$).

For treatment 3, Bacillus spores were activated at 30°C during 1 hour and subsequently added to the Artemia metanauplii suspension at 25°C. After 30min a gradual temperature decrease was initiated in order to reach 6°C within 1 hour. Artemia metanauplii were further stored at 6°C in a cold storage unit for 5 hours.

Microbial samples were collected at the end of enrichment (21h), and for treatment 3, additional samples were taken after 1h and 6h of cold storage. Microbiota composition was analyzed using 16S rRNA gene sequencing, enabling taxonomic classification and relative abundance profiling.

## Results

Microbial analysis revealed a significant reduction in Proteobacteria, the phylum that includes *Vibrio* spp., in both probiotic-treated groups, compared to the control. Concurrently, an increase in Firmicutes—containing the *Bacilli* spp.—was observed, indicating a shift toward a more favorable microbial profile. Furthermore, during the cold storage period, the relative abundance of Firmicutes continued to rise, while the Proteobacteria declined.

These shifts suggest that both probiotic strategies can effectively modulate the microbial community of *Artemia*, with potential implications for larval health.

## Discussion

The ability to influence the microbiota of live feed offers a valuable tool for hatchery managers aiming to reduce disease risk and improve larval performance. The observed reduction in Proteobacteria (including *Vibrio* spp.), combined with the enrichment of beneficial taxa, supports the use of targeted probiotic protocols as part of integrated biosecurity strategies.

Treatment 3, involving post-harvest contact with activated *Bacillus* spores, appears particularly promising due to its rapid and effective microbial modulation, even under cold storage conditions. This flexibility could facilitate operational integration in commercial hatcheries.

## Conclusions

Probiotic enrichment of *Artemia* using Sanolife® MIC-F demonstrates a clear potential to modulate microbial communities in favor of beneficial taxa, reducing the prevalence of potentially pathogenic bacteria. This approach contributes to enhanced biosecurity and supports the sustainability of intensive marine larviculture systems.

# HEART OEDEMA IN NEWLY HATCHED LARVAE: A TRANSCRIPTOMIC INSIGHT

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**Introduction**

Aquaculture continues to face challenges in producing high-quality larvae and early developmental deformities, often leading to high mortality and reduced production efficiency (Boglione et al., 2013; Chandra et al., 2024), are among major concerns. Variation in the type and occurrence of deformities across species, populations, and even within families complicates efforts to study and understand their underlying causes. While deformities are generally linked to genetic, biological, and external factors, those occurring immediately after hatching are more likely to result from genetic or non-genetic parental influences (Adrian-Kalchhauser et al., 2020). Among others, heart oedema is one of the most common deformities and is often expressed as a lethal trait. In Eurasian perch (*Perca fluviatilis*), this condition, marked by fluid build-up around the heart, is often observed in larvae obtained from spawners grown in recirculating aquaculture systems (RAS), impairing their cardiovascular function (Alix et al., 2017). Despite its prevalence, its underlying mechanisms remain poorly understood. This study aims to compare the within-family transcriptomic profiles of normal-shaped newly hatched larvae with those exhibiting heart oedema, offering insights into its molecular basis and potential strategies for improving broodstock management and larval quality.

**Materials and Methods**

Eurasian perch larvae from 8th-generation domesticated broodstock reared in RAS were used. Eggs were fertilized *in vitro* using cryopreserved milt and incubated under controlled conditions. On the day of hatching, larvae showing heart oedema and normal-shaped siblings were selected from 5 different families. Total RNA was extracted from each family and phenotype, and sequenced (10 libraries, 150 bp paired-end, 40M reads per library). Differentially expressed genes analysis was performed using DESeq2. Gene ontology enrichment (GO) was conducted with ShinyGO. Heart-specific genes were identified using PhyloFish database and further validated by RT-qPCR.

**Results**

Heart oedema has been found to be a lethal trait in Eurasian perch. Transcriptomic analysis revealed 507 differentially expressed genes (DEGs; $p < 0.05$, $\log_2 FC > 1$). GO analysis of DEGs indicated strong enrichment in metabolic regulation, stress response, and DNA repair. Seventeen heart-specific DEGs ($\tau > 0.85$) were selected for RT-qPCR validation. Their expression was tracked across key embryonic developmental stages to assess their role in cardiac development. Among these, *arl14, hbz* and *c18orf21* has been positively validated with qPCR. Developmental profiling showed lack of these genes in unfertilized eggs, low expression levels shortly after fertilization, with a noticeable increase around 100 hours post-fertilization (tail detachment stage), followed by a sharp rise at approximately 7 days post-fertilization, corresponding to the eyed-egg stage.

**Discussion**

This study provides novel insights into the processes underlying larval cardiac deformities and offers a foundation for developing strategies to mitigate mortality and improve aquaculture outcomes. The presence of both heart-oedema and normal larvae within the same family, sharing the same genetic background, suggests complex interactions between parental gametes, which we term 'parental dispute' (Debernardis et al, 2024). This highlights the crucial role of gamete-derived non-genetic factors underlying heart oedema. Additionally, the genes validated through qPCR emerge as strong candidate markers for further investigation into cardiovascular development and stress responses in fish embryos. Profiling their expression at critical embryonic stages revealed that their expression starts after zygotic genome activation and is associated with further heart morphogenesis (Alix et al., 2015). Our results suggest that disruptions in gene regulation during these stages may contribute to heart oedema.

Overall, these findings enhance our understanding of larval deformities, emphasizing the role of non-genetic inheritance factors in shaping larvae quality in E. perch.

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# USE OF SUGAREX AQUA TO MITIGATE NEGATIVE IMPACTS OF LOW-PALATABILITY AND MEDICATED FEEDS IN ATLANTIC SALMON (*Salmo salar*)

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## Introduction

Dietary changes can negatively affect feed intake in fish, leading to suboptimal performance. To address issues of palatability, various feed additives have been explored. Sugarex AQUA is a proprietary neohesperidin dihydrochalcone (NHDC) formulation with enhanced solubility and sensory properties. Research in other livestock has shown that NHDC, can enhance feed intake and improve growth performance. However, data on its effects in aquaculture remain limited. This study aimed to evaluate the impact of Sugarex AQUA on feeding behaviour and performance of Atlantic salmon (*Salmo salar*) when added to low-palatability or medicated diets.

## Materials and Methods

Groups of 30 162g Atlantic salmon (*Salmo* salar) were held in 30 500 L holding units forming part of a recirculating aquaculture system (RAS) with full strength sea water having a target temperature of 13 $\pm$ 1$^{o}$C and a with a light/dark cycle of 12/12 h. The trial was split into four two-week periods and the fish in each holding tank were weighed in bulk every two weeks. Feeding behaviour was assessed using a fixed scoring system.

Six treatments were tested with five replicates per treatment. The first set of treatments consisted of a control diet formulation with or without supplemental Sugarex AQUA (CD and CDS respectively). The second set of treatments consisted of a low-palatability diet with or without supplemental Sugarex AQUA (LP and LPS respectively). The final set of treatments were similar to the first two treatments except during the second two-week period during which an antibiotic (oxytetracycline) was added to the feed with or without supplemental Sugarex AQUA (TM and TMS respectively). Sugarex AQUA concentration in the supplemented diets was 0.6 g/kg, equivalent to an inclusion level of 18ppm NHDC.

Data was checked using Shapiro-Wilk and Levene's tests. T-tests or Mann-Whitney U tests (https://www.statskingdom.com) were used to compare similar diets ($p < 0.05$).

## Results

No significant differences in fish weight or specific growth rate (SGR) were observed between fish fed diets CD and CDS. Feed intake was slightly higher in CDS-fed fish, and feed conversion ratio (FCR) was consistently improved. Sugarex AQUA inclusion gave an SGR and FCR improvement of 8% and 4%, respectively. Sugarex AQUA had a more pronounced effect on fish fed the LP diets, significantly improving fish growth in Periods 1 to 3. Feeding behaviour and feed intake were also significantly higher in LPS-fed fish. In Periods 2 and 3, fish fed the TMS diet clearly outperformed those on the TM diet, showing significantly higher SGRs. Although the difference in SGR was not statistically significant in Period 4, the TMS group maintained a stronger overall growth, with a SGR 26% higher than the TM group. From Period 2 onwards, feed intake and SFR were consistently better in the TMS group, remaining significantly higher through to the end of the trial. Additionally, fish fed TMS achieved better feed efficiency, with FCR being consistently lower than the TM group in all periods, most notably in Period 3, resulting in a 13% overall reduction in FCR compared to the TM diet.

## Discussion

There were no clear benefits of adding Sugarex AQUA to the control diet. Conversely, Sugarex AQUA had a clear positive effect on feed intake and overall performance when included in the low-palatability diet for fish. The greatest impact was observed in Periods 1 and 2, diminishing over time, likely due to adaptation to the taste of the low-palatability formulation.

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The results related to Sugarex AQUA use alongside antibiotics were also clear. No significant differences were observed in Period 1 but, when antibiotics were introduced in Period 2, feed intake and SGR significantly increased in the Sugarex AQUA-supplemented group. It was expected that, once antibiotics were discontinued, the differences between the two treatments would disappear. However, significant differences in feed intake and performance persisted through Periods 3 and 4 and across the entire trial period. These results suggest that antibiotic use may have had a lasting impact on fish performance even after treatment ceased. This effect could be attributed to changes in gut microbiota (Dhanasiri et al., 2024; Monticelli et al., 2024), although this was not investigated in this study. Over the full trial period, fish fed the medicated diet (TM) were significantly smaller and exhibited lower growth than those fed either the control diet (CD) or the Sugarex AQUA-supplemented control diet (CDS). On the other hand, NHDC has been shown to positively influence gut morphology and microbiota (Daly et al., 2016; Wang et al., 2024), which may have helped mitigate the negative effects of the TM diet. As a result, by the end of the experiment, there were no significant differences in SGR and FCR between fish fed TMS and those on the control diets with or without Sugarex AQUA.

**Conclusions**

The findings of this study indicate that Sugarex AQUA can be beneficial in two key situations commonly encountered in aquaculture:

- Dietary Changes: Sugarex AQUA can help maintain feed intake and performance when feed formulations change due to ingredient availability, cost fluctuations, etc.
- Antibiotic Use: Sugarex AQUA supplementation can counteract the reduction in feed intake associated with antibiotic treatments.

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# A HOLISTIC AND COMPARATIVE STUDY OF THE MICROBIOME OF RAS OF SENEGALESE SOLE (*Solea senegalensis*) AND OF IMTA-RAS WITH SEA LETTUCE (*Ulva ohnoi*)

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## Introduction

Integrated Multitrophic Aquaculture in Recirculating Systems (IMTA-RAS) with macroalgae can mitigate the environmental impacts of fish production. *Ulva* spp. efficiently absorb nutrients, such as nitrate and phosphate, and transform them into biomass for food, feed or bioactive molecules. Moreover, the introduction of *Ulva* spp. can potentially modify the microbiome of the whole system. It is known that *Ulva*'s laminar blade offers support for beneficial bacteria, such as *Phaeobacter* spp., antagonists of fish pathogens. To understand the impact of *Ulva* on the system's microbiome, a holistic analysis of the microbiome was conducted in a commercial-scale pilot scale system.

## Materials and Methods

Two sequential trials were performed: one with sole fish (*Solea senegalensis*) in a RAS, and a second integrating sole with sea lettuce (*Ulva ohnoi*) (IMTA-RAS). The global microbiome of RAS and IMTA-RAS were compared using 16 rRNA metagenomic approach, which included samples taken from sea lettuce, sole (skin, gills, distal intestine mucosa), feed, , and the culture system (tank walls, biofilter and recirculating water). The study also included the analysis of parameters of the fish (growth, welfare markers and quality), the *Ulva* (growth and biochemical composition) and water (physicochemical parameters, nutrients) in order to establish, in future work, possible correlations.

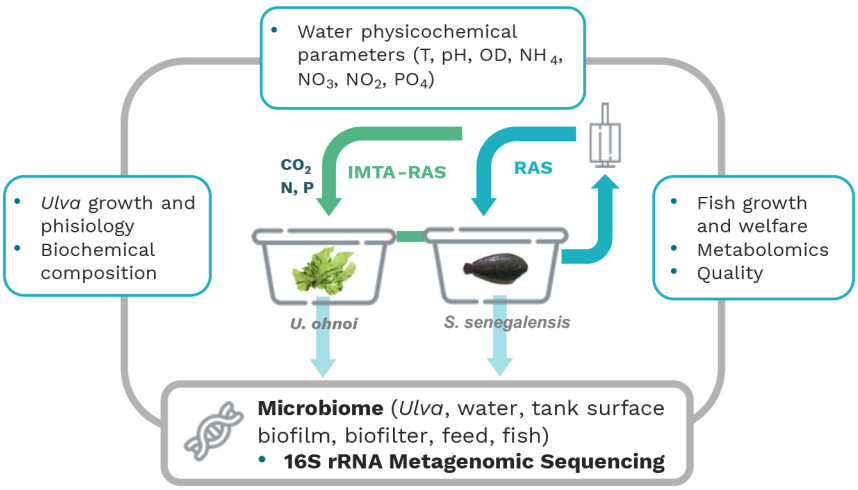


Fig. 1: Experimental design

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## Results

The results showed that the introduction of *U. ohnoi* significantly affects the microbiome of the system, including the one in the recirculating water, in the biofilter and in the biofilms of the tank walls, by altering both diversity metrics and taxonomic composition. Water samples taken before the biofilter consistently showed higher richness and diversity than those collected after ther biofilter, regardless of system type. Over time, beta diversity analyses revealed differences between RAS and IMTA-RAS in water microbiome at all timepoints and across compartments. Water in IMTA-RAS system was enriched in genera such as *Ruegeria*, *Vibrio*, *Maribacter*, *Polaribacter*, and *Sulfitobacter*. Some of these genera are associated with probiotic traits, biofilm stabilization, or antagonism against fish pathogens, suggesting potential health benefits. In the biofilter, microbial composition shifted temporally. At initial stages, *Roseovarius* dominated in RAS, while IMTA-RAS showed higher abundances of *Maribacter*, *Sulfitobacter*, and members of *Bdellovibrionaceae*. By the final sampling point, RAS was characterized by the presence of *Hyphomonadaceae*, whereas IMTA-RAS continued to support *Rubidimonas* and *Sulfitobacter*, the latter being consistently present and linked to sulfur metabolism and antimicrobial properties. Biofilms on fish tank walls also responded to *U. ohnoi* introduction. IMTA-RAS biofilms showed higher microbial richness and diversity, especially in early stages. *Nitrospira*, a genus involved in nitrogen cycling and known to contribute to biofilm stability, was dominant in IMTA-RAS, potentially enhancing system resilience. In contrast, RAS biofilms became dominated by *Thiothrix* at the final timepoint, a genus frequently linked to sulphide production and biofouling issues, which may indicate undesirable microbial development in RAS. These microbial shifts indicate that the inclusion of *U. ohnoi* may favour a more stable and functionally beneficial microbial community, potentially contributing to improved system health and pathogen resistance. Regarding the effect on fish, the analysis of the microbiome of *Solea senegalensis* is still ongoing. However, recent published results (Molecules 2025, 30(12), 2518) of metabolomic analysis of *S. senegalensis* muscle from those experiments revealed enhanced energy metabolism in IMTA-RAS systems, suggesting increased overall metabolic activity. These findings are promising, and upcoming microbial analysis may help establish a link between the systemic microbial environment and fish physiology, offering insights into the functional benefits of integrated multitrophic aquaculture.

## Conclusions

The integrated analysis of the results will allow an understanding of the changes in the microbiome of the systems and demonstrate the potential effect of IMTA-RAS on fish health and welfare. By operating IMTA-RAS systems, the aquaculture sector could diversify, decrease its environmental footprint, and producing in a healthier way.

## Acknowledgments/Funding

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# COMBINED THERAPEUTIC STRATEGIES AGAINST *Piscicketsia salmonis* IN *Salmo salar*: EVALUATION OF THE SYNERGISTIC POTENTIAL BETWEEN FLORFENICOL AND A PHYTOGENIC ADDITIVE

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The botanical product evaluated in this study represents an innovative and sustainable formulation as an alternative to the use of antibiotics in aquaculture. Its combination of organic acids and plant extracts has demonstrated effective antimicrobial properties, as well as supporting fish welfare, ensuring the quality of the final product and reducing the risk of bacterial resistance.

To evaluate its effectiveness, the administration of Florfenicol at 20 mg/kg for 10 days was compared with a reduced dose of Florfenicol (10 mg/kg) combined with the natural product, in Atlantic salmon (*Salmo salar*) experimentally infected with *Piscirickettsia salmonis*. The challenge was performed by intraperitoneal inoculation using two genetically distinct strains: LF-89 and EM-90, recognized for their difference in virulence and pathological patterns. Variables such as mortality, bacterial load, immunological parameters and histopathological indicators were evaluated.

Histopathological analyses revealed that the LF-89 strain predominantly produces kidney and liver lesions, while EM-90 generates a more systemic effect, with hemorrhages in multiple organs. Variables such as mortality, immunological markers, bacterial load and general health indicators were evaluated.

During the test, the fish maintained an intake greater than 1.5% of their body weight and no relevant biometric alterations were observed. Clinical signs appeared between days 20 and 26 post-inoculation in the positive control group, with a cumulative mortality of 40% at day 37. In contrast, both the botanical + Florfenicol combination and Florfenicol alone reduced mortality to 6.7%, equaling the negative control.

Both treatments also achieved a significant reduction in bacterial load. The combination with the botanical product at half dose of the antibiotic obtained an efficacy comparable to treatment with Florfenicol at full dose. This suggests a synergistic action that enhances the effect of the antibiotic and stimulates the immune system. Complementing these results, a field study conducted on *Oncorhynchus kisutch* showed that the exclusive use of the botanical product significantly decreased the scores of oxidative stress, inflammation and deficient immunocompetence. In addition, better integrity of the skin, fins and mucus, as well as a reduction in external injuries.

In conclusion, the botanical product presents a promising and sustainable approach to the control of *Piscirickettsia salmonis* in Atlantic salmon, allowing to reduce the use of antibiotics by up to 50% without compromising the efficacy of the treatment.

# EVALUATION OF A BOTANICAL FORMULATION AS AN IMMUNOPROTECTIVE AND ANTIMICROBIAL STRATEGY IN *Salmo salar* CHALLENGED WITH *Piscirickettsia salmonis*

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Salmon Rickettsial Septicemia (SRS), caused by *Piscirickettsia salmonis*, is one of the main bacterial diseases in Chilean salmon farming. It generates high mortality rates and production losses, which has driven the search for more sustainable therapeutic alternatives.

This study evaluated the efficacy of a broad-spectrum natural botanical formulation designed to inhibit bacterial growth through multiple mechanisms: disruption of quorum sensing signaling, alteration of membrane integrity, and inhibition of protein and nucleic acid synthesis. In addition, it sought to enhance the fish's immune defenses, as well as reduce inflammation and oxidative stress.

The trials were carried out in farming centers in southern Chile. Groups of *Salmo salar* were fed the formulation for 30 days and then challenged with *Piscirickettsia salmonis* (strains LF-89 and EM-90) for another 30 days. Mortality rates, prevalence of infection, and physiological, histological and molecular analyses were carried out.

The treated fish showed a 64% reduction in infectious mortality compared to the control group. Significant activation of immune markers related to salmon rickettsial septicaemia (SRS), including CD4, CD8, IFN-γ, TNF, IL-1β, IL-10, lysozyme, and transferrin, was evidenced, indicating a potentiated adaptive immune response. Likewise, a decrease in oxidative stress was observed, reflected in lower activity of antioxidant enzymes in NFEL2, SOD3, CAT, GPX markers and in the reduction of the expression of genes associated with epithelial damage in the intestine, skin and gills, reflected by MMP2, MMP13, COL1A1.

From the histological point of view, the treated fish had higher intestinal villi height, less inflammation in enteric mucosa and greater density of mucosal cells in the skin, which suggests an improvement in the absorption capacity and in the barrier function against external agents (pathogens and inflammatory). In addition, a significant decrease in the general histopathological score was observed**,** with marked reductions in the levels of necrosis, inflammatory infiltration, and tissue damage, especially in the liver, kidney, and spleen.

Positive differences were observed in terms of production parameters, such as a 17% increase in growth and -7% FCR (feed conversion rate).

Taken together, these findings support the use of this botanical formulation as an effective and sustainable strategy for the management of *Piscirickettsia salmonis*. Its use significantly reduces mortality and strengthens the overall health of the fish, while minimizing the need for antibiotics and the associated risk of antimicrobial resistance in salmon farming.

# EVALUATION OF FRESHWATER IMMERSION TREATMENTS FOR FUNGAL AND FLAVOBACTERIUM INFECTIONS IN FISH: A WELFARE PERSPECTIVE

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Fungal infections and flavobacteriosis are significant health challenges affecting *Salmo salar* in aquaculture systems, during freshwater phases. These pathogens can compromise fish welfare, cause lesions, and increase mortality if not promptly managed. Salt baths, Bronopol and Formaline are commonly used as a curative and preventive treatment, yet their impact on animal welfare remains underexplored. Understanding the effectiveness and welfare implications of such treatments is critical for sustainable fish health management. This study evaluated the effect on animal welfare and health of two conventional preventive and curative treatments in fresh water, Salt at 2%, Formalin at 150 ppm and 40 ppm of Bronopol with AQBiocare Plus®, a naturally formulation derived of plants with antimicrobial broad spectrum with antioxidant and anti-inflammatory properties. The in vitro and in vivo studies in Atlantic salmon demonstrated the antibacterial efficacy against key pathogens, including Flavobacterium and Saprolegnia. In vitro studies validated through absorbance and MIC assays that AQ Biocare Plus® shows strong antimicrobial activity lasting 24 to 48 hours during immersion treatments and on fungal the doses of inhibiting to Saprolegnia were 50 ppm.

Beyond its antimicrobial properties, AQBiocare Plus® enhances innate immune responses, and takes care of the production and quality of the skin mucus, promoting disease resistance. It significantly strengthens natural immunity by improving cutaneous, mucosal, and serum immune parameters while upregulating immune-related genes. At the molecular level, it enhances inflammatory, immune, antiviral, and antibacterial responses while mitigating oxidative stress by reducing and scavenging free radicals. The markers of anti-inflammatory AqBiocare showed a stable response (IL1b, IL10, TNFa,) and tissue remodeling indicators (Col1A1, MMP2, MMP13) suggested a reduced skin damage compared with Formaline and Salt. The formulation also demonstrates superior control over reactive oxygen species (ROS), resulting in reduced oxidative stress, with lower activity of NFEL2, SOD3, GPX, and CAT, alongside high lysozyme levels, indicating enhanced bacterial protection. Fish treated with AQBiocare Plus® showed improved physiological balance, with up to 25% lower cortisol and 18% lower glucose compared to Salt or Formalin. Welfare biomarkers remained stable even under smoltification stress, indicating enhanced stress resilience.

Histologically, AQ Biocare Plus® increases mucous cell density in the skin, improves the quality and the quantity of the mucus, strengthening the fish's natural barrier, it has better effect on Lizozime. Welfare analyses, including serotonin, cortisol, lactate, glucose and osmolality measurements, highlight its positive impact on fish well-being.

These comprehensive benefits position AQ Biocare Plus® as an innovative and sustainable alternative in salmon farming, effectively protecting fish health, enhancing productivity and improving animal welfare.

# INNOVATIVE NON-PHARMACOLOGICAL APPROACHES FOR MANAGING *Caligus rogercresseyi*: IMPLICATIONS FOR THE HEALTH AND WELFARE OF ATLANTIC SALMON

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Global aquatic animal production has increased substantially since 1950, reaching 185 million tonnes in 2022, with aquaculture contributing over 51% of total output. In Chile, Atlantic salmon (Salmo salar) dominates the export-oriented aquaculture industry but faces major health challenges, especially caligidosis caused by the ectoparasite Caligus rogercresseyi. This parasite disrupts immune, neuroendocrine, and metabolic functions, reducing fish welfare and causing economic losses. Concerns over drug resistance, environmental impact, and animal welfare have driven interest in sustainable, non-pharmacological alternatives.

This study assessed the antiparasitic efficacy and physiological benefits of Rosseus Plus®, a botanical supplement based on essential oils and plant extracts, administered via therapeutic baths and water exposure. In vitro assays showed 93–100% effectiveness against adult C. rogercresseyi, while field trials using wellboats achieved parasite reductions of 98% in adults and 85% in juveniles. Waterborne delivery enhanced immune responses, lowered stress markers, and improved the intake of the feeding and the growth.

Molecular, histological, and physiological analyses revealed significant reduction in acute and chronic stress, mitigation of oxidative stress, lowering inflammation in gill and skin. Treated fish maintained stable cortisol levels, preserved lysozyme activity, uncompromised immune response, balanced osmoregulation and consistent serotonin concentrations, indicating improved neuroendocrine balance and welfare. The inclusion of antioxidant, serotonergic biomarkers provides a robust, science-based framework to monitor fish resilience and welfare, offering precise evaluation of health and treatment effects.

These findings support Rosseus Plus® as a safe, effective, and environmentally sustainable strategy for integrated parasite management in Atlantic salmon aquaculture, promoting welfare-focused and responsible production practices.

# TEMPERATURE EFFECT ON MICROBIAL COMMUNITIES AND CULTURED PIKEPERCH LARVAE UNDER COMMERCIAL CONDITIONS

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**Introduction**

Pikeperch (*Sander lucioperca* (L., 1758)) is a promising candidate for the diversification of inland aquaculture in Europe, although the production of sufficient fingerlings remains a bottleneck. The most critical stage of life during fish production is the larval period. Numerous factors regulate the larval survival, including environmental parameters (e.g. water quality, water temperature, and photoperiod), nutritional – (e.g. feeding frequency, weaning strategy), and population factors (e.g. stocking density).

The microbial environment of fish larvae is an additional factor that may determine the production success, since the microbes interact with the larvae at different levels. Microbes with an external origin (water and feed) will colonise the digestive tract of the fish and therefore influence the health status of the animal. The microbial community from the intestine of teleosts, namely fish gut microbiome, is an important element, ensuring the proper growth and health. However, in order to ensure economic production, the animals are confronted with various challenges. These include switching from live feed to commercial dry feed, called the weaning process. The microbiome changes abruptly with the habituation to dry feed. The status of the environmental and gut microbiome during this process under different feeding regimes and environmental conditions is still not evident in pikeperch. Information about microbial communities could provide key aspects related to the fish larvae production.

We aimed to assess the effect of water temperature on fish performance and the microbial communities of the system, and to describe the microbial communities present in the water, the fish gut, and the feed during the initial stages of larval rearing, after first feeding.

**Material and methods**

In a 47-day larval rearing experiment, we investigated the differences between two temperature regimes (16 °C vs. 20 °C) in two identical recirculated systems. The 500-litre tanks were used in triplicate, combined with high stocking densities (150 larvae/litre), and a 24-hour photoperiod. For the microbiological investigation, samples of water and fish larvae were collected in triplicates at three time points (24, 34 and 47 days post-hatch: T1, T2, and T3). After DNA extraction, we sent the samples to Edinburgh Genetics Limited for whole genome sequencing and metagenomic sequencing analysis. Data and statistical analysis were conducted using various diversity indices, PCoA, Bray-Curtis ANOSIM, and relative abundances of taxa. To assess larval performance, we measured growth parameters (body wet-weight, total length), survival rate, swim bladder inflation rate, and the occurrence of jaw and spine deformities. Statistical differences were evaluated ($p < 0.05$) using one-way ANOVA and Mann-Whitney U Test (SPSS 29.0, IBM Co., Armonk, NY, USA).

**Results and conclusion**

At 20 °C, the larvae performed significantly better in terms of final wet-weight, final total length, and swimbladder inflation rate compared to the 16 °C system. However, we found no significant differences regarding the survival and deformation rates.

We observed a temporal variation in water microbial community structure and composition. Regarding the two different temperatures (16 °C vs. 20 °C), no statistically significant differences were detected in either the water microbial community or the fish microbiome. In the rearing water, *Vibrio* sp. and *Pseudoalteromonas* sp. were the dominant pathogens at T1, but their abundance decreased at T2 and T3. At T2 and T3, the dominant pathogen was *Aeromonas* sp.

*(Continued on next page)*

In conclusion, higher rearing temperatures can enhance the growth and performance of pikeperch larvae without significant negative effects. Further investigations are required to address the non-significant, yet visible differences at 20 °C, such as higher deformity rates and a relatively higher abundance of *Vibrio* sp. in the larvae gut during the live feed period.

The European Fisheries Fund (EMFAF) and the Ministry of Agriculture and Environment from Mecklenburg-Western Pomerania funded this study as part of the project Fish pathogens from fish farms and waters of the Northeast German Lake District (project number MV-II. 12-LM-03).

# POLYMERIC AND EMULSION ADJUVANTS FOR *Streptococcus agalactiae* GHOST VACCINE IN NILE TILAPIA

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**Introduction**:
*Streptococcus agalactiae* is a major threat in tilapia aquaculture. This study evaluated the safety and efficacy of a *S. agalactiae* ghost vaccine (SAGV) formulated with novel adjuvants, Montanide™ GEL 02 (polymer) and Montanide™ ISA 763B VG (emulsion).

**Materials and Methods**:
For safety, 10 fish/group (~50g) were injected intraperitoneally with 0.2 ml of: PBS (control), SAGV alone (1 x $10^7$ CFU/ml), SAGV with Montanide™ GEL 02, or Montanide™ ISA 763B VG. Fish were observed for one week for adverse effects and necropsied at 4 and 8 weeks post-vaccination to evaluate chronic side effects. For efficacy, 90 fish/group (~ 50g) were injected intraperitoneally with 0.2 ml of the same formulations. Serum bactericidal activity and anti-*S. agalactiae* IgM titers were measured weekly for two months. Eight weeks post-vaccination, 45 fish/group were challenged with virulent *S. agalactiae* (1 x $10^8$ CFU/ml). Dead fish were collected daily, and tissues were collected for bacteriological examination to confirm *S. agalactiae* as the cause of death.

**Results**:
Safety assessment showed limited oily vaccine residue in the Montanide™ ISA 763B VG group due to the deposit effect inherent to oily adjuvants that allows slow antigen release. No aqueous vaccine residue was observed in the Montanide™ GEL 02 group. Both adjuvants significantly enhanced IgM antibody titers from 2 to 8 weeks post-vaccination ($p < 0.01$). Montanide™ GEL 02 and Montanide™ ISA 763B VG significantly improved protection against *S. agalactiae* challenge compared to SAGV alone ($p < 0.05$), with survival rates of 93.33% and 88.89%, respectively.

**Conclusion**:
The aqueous adjuvant Montanide™ GEL 02 and the oily adjuvant Montanide™ ISA 763B VG, are safe and effective for injectable *S. agalactiae* ghost vaccines in tilapia. They enhance both the humoral immune response and protection against bacterial challenge.

# VERMI-TEA PRODUCTION FROM FISH WASTE: A SUSTAINABLE APPROACH TO NUTRIENT RECOVERY IN AQUAPONICS

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## Introduction

Aquaponics systems present a promising solution for sustainable food production by integrating aquaculture and hydroponics. However, managing nutrient cycling and waste reuse remains a critical challenge. One innovative solution involves the incorporation of a vermi-trio reactor that utilizes fish sludge and plant waste for nutrient recovery and ammonia removal. This study explores the development and integration of such a reactor into an aquaponics setup, emphasizing the conversion of system-derived organic waste into valuable biofertilizers—vermicompost, vermiwash, and vermi-tea superior to its conventional counterparts (i.e. vermicompost system etc.). This closed-loop approach not only optimizes resource use efficiency but also reduces environmental impact and the dependency on synthetic inputs, making aquaponics more sustainable and economically viable.

## Materials and methods

The vermi-trio reactor was conceptualized focusing on a vertically stacked tray design to conserve space and enhance process efficiency. The structure was fabricated using durable wooden trays, selected for their cost-effectiveness and structural stability. To improve resistance against decomposition during composting, each tray was coated with two layers of plastic-based paint. The reactor accommodates the efficient transformation of fish sludge and plant waste into biofertilizers through vermicomposting. To ensure optimal functioning, the system is operated under controlled conditions suitable for *Eisenia fetida*, the earthworm species used for decomposition. An automated sensor system monitors and regulates environmental conditions, maintaining a temperature of 25–30°C and relative humidity of 50–70%, which are critical for efficient microbial activity and earthworm performance.

## Results

A small scale earthen pot setup was used for producing vermicompost using fish sludge and plant waste from the aquaponics system. The resulting vermicompost was nutrient-rich, containing phosphorus (0.455%), organic carbon (21.74%), and manganese (494 ppm), exceeding the nutrient concentrations typically observed in traditional cow dung-based vermicompost, while maintaining comparable levels of nitrogen, potassium, iron, and magnesium. This demonstrates the fish sludge vermicomposting efficiency in nutrient recovery and its potential as a sustainable alternative for organic waste management in aquaponics. By facilitating the reuse of system-generated waste, the vermin-tea reactor can enhance nutrient availability for plants, mitigates ammonia accumulation, and reduces reliance on synthetic fertilizers. This closed-loop design promotes resource efficiency and environmental sustainability in soilless farming systems. Future research will focus on testing of vermi-tea reactor and optimizing nutrient recovery processes and assessing long-term impacts on both plant and fish productivity to evaluate the scalability and commercial viability of this technology across diverse agricultural contexts.



Fig. 1. Fabricated vermi-trio reactor for vermi-tea recovery from aquaponics waste.

# AQUATIC MOSS MATS AS ALTERNATIVE BIOFILTER MEDIA FOR AQUAPONICS

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**Introduction**
Conventionally, inert media such as plastic, ceramic or zeolite are conventionally used for wastewater biofiltration. However, these media need activation by microorganisms, and differentiate essentially based on their surface/mass ratio since biofiltration entirely occurs at the surface biofilm. The use of biodegradable media may enhance the sustainability of the system, while avoiding generation of decomposition-related pollutants. In this respect, due to their surface extension, peculiar microbiota and structural resistance, aquatic mosses appear to be very good (and innovative) supports for biofilters.

**Materials and Methods**
In this study, we evaluated aquatic moss mats as alternative mats for the biofiltration of aquaculture or aquaponic effluents. Two moss species [1], *Taxiphyllum barbieri* and *Leptodictyum riparium,* were obtained in semi-axenic form (Green Greener srl Policoro, Italy) and tested for their contribution on N metabolism, by evaluating i) TAN, $NO_2$ and $NO_3$, ii) competition with hydroponic cultivation by comparative application, and iii) microbiota association by 16S rRNA metabarcoding. A direct comparison with a classic biofilter was performed in a complete RAS populated with Apulian naturalized [2] Nile tilapia (*Oreochromis niloticus*) obtained from Azienda Ittica Agricola Residence San Nazario srls (Lesina, Foggia, Italy).

**Results**
Due to their extraordinary surface extension, mosses performed very well in N metabolization without preliminary activation. Competition with plants grown in a hydroponic culture configuration was not significant, which may have positive aspects. Mosses microbiota was peculiar and did not favor parasites or pathogens. Therefore, for their structural resistance, aquatic mosses appeared a very good support for biofilters demonstrating equivalence in real conditions with inert media. However, the quantity of mosses required can be several times less than any competing media.

**Conclusions**
Aquatic moss biomasses showed to be valuable and efficient biofilter media applicable to aquaculture, hydroponics and aquaponics.

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# EGG POWDER AS A FUNCTIONAL INGREDIENT: EXPLORING ITS ROLE AS A NATURAL AND ECONOMICAL CHOLESTEROL ALTERNATIVE FOR SUSTAINABLE SHRIMP AQUACULTURE FEED STRATEGIES

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Cholesterol is an indispensable nutrient in crustacean aquaculture, particularly for species like the Pacific white shrimp (*Penaeus vannamei*), which lack the metabolic pathways for de novo synthesis. Historically, marine ingredients like fish meal, commonly included in shrimp feeds, provided adequate levels of cholesterol. However, the drive towards sustainable aquaculture practices necessitates reducing reliance on fish meal due to cost, availability, and environmental concerns. Replacing fish meal with alternative protein sources, primarily plant-based, often results in diets deficient in cholesterol, compromising shrimp growth, survival, and overall health. Consequently, supplementation with purified, synthetic cholesterol has become a standard practice to meet the established dietary requirements (ranging from 0.05% to 1.0% depending on species and life stage, with 0.05-0.5% often cited for *P. vannamei*). While effective, synthetic cholesterol represents a significant cost factor in feed formulation, prompting a search for more economical and natural alternatives.

This review evaluates the potential of whole egg powder as a multifunctional ingredient capable of replacing synthetic cholesterol in shrimp diets. Whole egg powder is a nutrient-dense ingredient that provides high-quality protein, essential amino acids, energy-rich lipids, vitamins, and vital phospholipids. Moreover, it's manufactured through a gentle spray drying process that guarantees quality and high digestibility of proteins and essential amino acids. Crucially, it is also a natural source of cholesterol. This inherent cholesterol concentration suggests that incorporating egg powder into shrimp feed formulations could significantly contribute to, or potentially fully satisfy, the shrimp's dietary cholesterol requirement, thereby reducing or eliminating the need for expensive synthetic supplementation. The primary driver for exploring egg powder as a cholesterol alternative is economics. Egg powder is generally reported to be a more affordable ingredient compared to both high-quality fish meal and purified cholesterol. Replacing synthetic cholesterol with the cholesterol naturally present in egg powder could lead to considerable savings in feed production costs, enhancing the economic viability of shrimp farming operations. Beyond cholesterol, egg powder offers additional nutritional benefits. It provides a balanced amino acid profile, although potentially lower in certain limiting amino acids like methionine, lysine, and arginine compared to premium fish meals, requiring careful formulation. Furthermore, egg powder is rich in phospholipids, particularly phosphatidylcholine (PC), which are known to be essential for larval and juvenile shrimp growth, survival, and stress resistance. The phospholipid requirement for penaeid shrimp is estimated to be between 1.2-1.5%, often met by adding lecithin sources. Egg powder can contribute significantly to meeting this requirement.

Based on its natural cholesterol content, affordability relative to synthetic cholesterol, and additional nutritional contributions (protein, phospholipids, bioactive compounds), egg powder shows significant potential as a cost-effective alternative for cholesterol supplementation in *P. vannamei* diets. Its use could alleviate the economic burden associated with purified cholesterol while contributing to overall shrimp nutrition and health. However, successful implementation requires formulation to balance its different nutrients. Further research, including dedicated feeding trials with *P. vannamei*, is ongoing, and it's crucial to determine the optimal inclusion levels of egg powder for effective cholesterol replacement, evaluate its impact on growth performance and health, and fully realise its potential as a sustainable and economical ingredient in shrimp aquaculture.

# *Bacillus subtilis* SPORES MODULATE ANTIVIRAL RESPONSES IN RAINBOW TROUT

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## Introduction

In aquaculture, the use of probiotics in supplemented diets has been shown to be a suitable strategy to increase the immune status of fish and thereby reduce the impact of pathogens (Simón *et al.*, 2021). Specifically, the immunostimulatory effects of the probiotic microorganism *Bacillus subtilis* have been widely confirmed both *in vitro* and *in vivo* in many aquacultured species (Docando *et al.*, 2021; Vicente-Gil *et al.*, 2024). However, the potential effect of probiotic supplementation on antiviral capacities has been scarcely investigated in fish. Therefore, in this study, we aimed to address this gap using rainbow trout (*Oncorhynchus mykiss*) as a model species. Thus, fish were fed a diet supplemented with *B. subtilis* spores or a control diet during a long period (45 days) and the levels of transcription of a range of antiviral genes determined. In another experiment, after 30 days of feeding, spleen and head kidney leukocytes were isolated and exposed to two viral stimuli: Poly I:C - a synthetic analogue of double-stranded RNA (dsRNA) known to induce antiviral responses in rainbow trout cells (Simón et al., 2023) and heat-inactivated viral hemorrhagic septicemia virus (iVHSV). The levels of transcription of antiviral genes was then measured in these cells.

## Materials and methods

The basal diet used in all feeding trials was a commercial formulation (Skretting, Stavanger, Norway). To prepare the supplemented diet, lyophilized spores of the probiotic bacteria *B. subtilis* dissolved in 0.9% NaCl saline solution, were thoroughly mixed with the basal feed pellets. The spore concentration used was 1 x $10^{10}$ spores/kg of feed. Healthy rainbow trout (*Oncorhynchus mykiss*) were fed either the control or the enriched diet once a day at a rate of 2% body weight per day for 45 or 30 days, for the *in vivo* and the *ex vivo* trial, respectively. In the first experiment, fish were euthanized by benzocaine overdose (50 mg/ml) and spleen and head kidney collected. The tissues directly placed in TRI Reagent solution for RNA extraction. In the second experiment, kidney and spleen were collected from sampled fish for the isolation of total leukocyte populations. To this aim, tissues were mashed through a 100 µm nylon mesh and placed onto 30/51 % Percoll gradients, following the protocol described by Vicente-Gil *et al.* (2024). Total head kidney and splenic leukocytes were then diluted in L-15 supplemented with antibiotics (1% penicillin/streptomycin) and 2% fetal calf serum and seeded onto 24-well plates at concentrations of 4 x $10^6$ and 1 x $10^7$ cells/well, respectively. At this point, either 50 µg/ml of poly I:C or heat inactivated VHSV (iVHSV, 1 × $10^6$ $TCID_{50}$/ml) were added and leukocytes incubated at 19ºC. Untreated cells were also included as controls for each fish. After 24 h, RNA was extracted with TRI reagent. cDNA was synthesized from RNA samples from both experiments and used to assess antiviral gene expression levels by real-time PCR, using the $2^{-\Delta Ct}$ method, where ΔCt is determined by subtracting the β-*actin* value from the target cycle threshold. Negative controls with no template and *minus* reverse transcriptase controls were included in all cases.

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## Results

In the *in vivo* trial, we observed limited transcriptional changes in response to the supplemented diet in both the head kidney and spleen. Particularly in spleen, only the expression of *tlr22* and *lpg2b* genes were significantly increased in fish fed *B. subtilis* spore-supplemented diet compared to the control group. Similarly, in head kidney, the transcriptional factor STAT3, known to be involved in antiviral pathways, was the only upregulated gene in fish receiving the supplemented diet. Comparable results were obtained in head kidney leukocytes from fish fed either the control or the *B. subtilis*-supplemented diets, and subsequently exposed to PolyI:C and iVHSV, showing no major differences in gene expression levels. However, spleen leukocytes from fish that received the probiotic-supplemented diet, exhibited upregulation of nearly all antiviral related genes studied, including interferons 1 and 2, TLRs and *stat3* upon viral stimuli at levels higher to those elicited by the virus in leukocyte cultures from fish fed the control diet. These results highlight the importance of diet composition in regulating the capacity of leukocytes to respond to viral stimuli. Specifically, the *B. subtilis*-supplemented diet appears to prime leukocytes, particularly in the spleen, to mount a stronger antiviral response, suggesting a promising prophylactic strategy for use in aquaculture.

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# BUILDING THERMAL RESILIENCE IN SALMONIDS THROUGH EXPERIMENTAL TESTING AND GENOMIC SELECTION

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Summer survival has become an increasingly critical focus for salmonid producers, as climate change has heightened thermal stress, leading to reduced growth and increased mortality. Improving thermotolerance through selective breeding offers a promising strategy to protect animal welfare and commercial returns. However, selecting for thermotolerance remains challenging due to the variability of environmental conditions and the invasive nature of heat challenge tests.

This study reports genetic parameters and improvement strategies for thermotolerance in a multi-generational Chinook salmon (*Oncorhynchus tshawytscha*) breeding program, based on both laboratory challenge tests and summer cage survival data. A laboratory-based thermotolerance challenge was conducted across three year-classes. Individuals were exposed to progressively increasing temperatures (15°C to 23.5°C) and maintained at elevated temperatures for over two weeks in recirculating systems. Traits recorded included feeding behaviour, weight and length (pre- and post-challenge), and time to death (TTD).

TTD displayed moderate heritability ($h^2$ ~0.4) across all year-classes, supporting its utility for selection. Incorporating genomic predictions via a medium-density, sequencing-based platform increased Estimated Breeding Value (EBV) accuracy by ~10% compared to pedigree-based methods. Importantly, thermotolerance traits measured in the lab were moderately and consistently correlated with summer cage survival within the same year-class ($r \approx 0.3–0.5$), validating the relevance of the challenge test to real-world performance.

These results highlight the value of combining validated laboratory phenotypes with genomic selection to address complex traits like thermotolerance. Integrating challenge-test performance with field-based survival data enables cumulative genetic gains, enhances fish resilience under warming conditions, and strengthens the sustainability of salmonid aquaculture.

# EMERGING PHENOTYPING METHODS FOR FEED EFFICIENCY IN ATLANTIC SALMON: FROM INTAKE TO LIPID UTILIZATION

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**Introduction**

Feed efficiency is a cornerstone of sustainable aquaculture, with the potential to reduce both production costs (Iversen et al., 2020) and environmental impact (Winther et al., 2020). However, direct phenotyping of individual feed intake and nutrient utilization in Atlantic salmon remains challenging at commercial breeding scales (de Verdal et al., 2017; Knap and Kause, 2018) so feed efficiency improvement is of great importance. Our aim is to use work done in pigs to formulate a logical framework for assessing the most useful component traits influencing feed intake and efficiency in farmed fish – either to identify traits that can together be used for genetic improvement of feed efficiency, or as substitute traits for feed intake recording. Improvement of gross feed efficiency in growing fish can be accomplished by selection for increased growth rate. However, the correlation of growth with feed efficiency is typically only modest, and hence there is room for further improvement of feed efficiency through methods other than growth selection. Based on a literature review we propose that the most effective additional methods are selection for reduced body lipid content and for reduced residual feed intake (RFI. To phenotype feed efficiency it is necessary to measure individual feed intake and calculate feed efficiency or identify traits in the bioenergetic pathway which contribute to variation in feed efficiency. King among bioenergetic traits are lipid deposition traits, lipid composition traits and their changes over time.

We present a suite of high-throughput, and scalable phenotyping methods aimed at capturing variation in feed intake, lipid metabolism, and overall feed efficiency across life stages. The objective of this study was to evaluate the feasibility, and genetic potential of these methods for phenotyping individual feed intake in Atlantic salmon.

**Materials and Methods**

We evaluated five novel phenotyping methods across ~2,000 Atlantic salmon from 23 full-sib families. Trials were conducted at the Norwegian Centre for Fish Trials (Ås, Norway) during the freshwater phase (50–100 g) and at Nofima's Centre for Recirculation in Aquaculture (Sunndalsøra, Norway) during the saltwater phase (500–2,000 g). On three separate occasions, fish were fed experimental diets containing 5% embedded metal micro-pellets and scanned using a calibrated metal detector.

During each trial, fish were fed to satiation in small family groups and scanned post-prandially using a semi-portable conveyor-based metal detector. At the third and final sampling, fish were euthanized humanely, gutted, and filleted. Feed intake was then confirmed through gut analysis using both metal detection and X-ray imaging. In parallel, tissue samples were collected for stable isotope and spectral analyses.

The data was analyzed with a linear mixed animal model using the DMU software (Madsen and Jensen, 2013), including fixed tank and sex, and a random additive genetic effect based on the genomic relationship matrix (G). Heritability ($h^2$) was estimated from variance components. The five phenotyping approaches evaluated were:

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Stable Isotope Analysis: $\delta^{13}C$ and $\delta^{115}N$ values were analyzed in fillet tissue to infer carbon and nitrogen assimilation as indicators of nutrient utilization and feed efficiency (Dvergedal et al., 2019, 2020). Family-level isotope ratios showed correlation with feed conversion ratio, providing a biochemical proxy trait for breeding. Metal Detector Scanning: A calibrated, conveyor-driven metal detector was used to non-invasively quantify individual feed intake based on postprandial field strength signals. The metal beadlets are Fish radio-opaque and were X-rayed post gutting to count markers in the gastrointestinal tract. Individual feed intake was estimated by calibrating marker count to feed weight (Difford et al., 2023; Ahmad et al., 2025b). Interactance Near-Infrared Spectroscopy (iNIR): Whole-body, fillet, and viscera NIR spectra were used to predict lipid content and feed intake through partial least-squares regression (PLSR) (Ahmad et al., 2025a). Raman Spectroscopy: Portable Raman probes were applied to fillets to assess fatty acid composition, focusing on omega-3 content (Lintvedt et al., 2022). This method is non-destructive and shows promise for high-throughput lipid phenotyping in breeding programs.

**Results**

Preliminary results from these trials will be presented. The study demonstrates the feasibility of several novel, non-invasive methods for phenotyping feed intake and efficiency in Atlantic salmon. While each method shows potential for application in selective breeding, differences in accuracy, heritability, and scalability were observed. These findings highlight both the promise and the current limitations of emerging phenotyping technologies, underscoring the need for continued research and development to improve their reliability, biological interpretation, and technology readiness level.

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# STRATEGIES FOR THE DEVELOPMENT OF INNOVATIVE MICROALGAE PRODUCTS TO SOLVE AQUACULTURE CHALLENGES

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The expansion of aquaculture is essential to meet the food security of the growing human population, which requires optimizing the sustainable development of the sector. Microalgae are a sustainable resource and are essential for the initial stages of development of marine fish larvae, being applied in green water technique, rotifer cultivation and enrichment of live feeds. Currently, the required volumes of microalgae exceed the production capacity within nursery infrastructures, which, combined with the high challenges of their production, leads to a high demand for commercial microalgae products. Although rotifers production and enrichment are broadly used for many decades in aquaculture, their nutritional requirements and metabolism are still poorly understood which impairs the development of high-performance products for these purposes. Some of the great challenges of microalgae products for aquaculture are the design according to the specific needs of end users and the design of formulations adapted to the species, aquaculture production systems and production methods. Additionally, products must be developed considering the major current challenges in aquaculture, such as the incidence of pathologies, water quality control and reduction of waste accumulation (product sedimentation). To solve the mentioned issues, Necton has developed 5 new sustainable microalgae-based products with added-value to fill these gaps. This work reviews the strategies of new microalgae products development to address the current challenges in marine fish nurseries production. Three products were developed in the new VitaBloom® product range, that combine microalgae and probiotics in powder for green water technique (Green water), rotifers nutrition (Rotifers+) and live feed enrichment (Enrich). Green water product is adapted for green water technique in open systems, Rotifers+ is developed for semi-continuous and batch rotifers production and Enrich is adapted for rotifers and artemia enrichment. Two new Premium *Nannochloropsis* sp. liquid concentrates were developed with low bacterial content and low microalgae agglomeration, adapted to different cultivation methods. PhytoBloom® ELITE formula (ELITEf) was developed for rotifers batch cultivation and PhytoBloom® RAS formula (RASf) was specifically designed for rotifers cultivation in the challenging Recirculation Aquaculture Systems (RAS) with semi-continuous production, and for green water technique. VitaBloom® range was obtained through the combination of different species of microalgae and yeast, to fulfil the nutritional requirements of rotifers and fish larvae and validated *in vivo* in rotifers and fish larvae cultivation (*Sparus aurata* and *Argyrosomus regius*). Premium PhytoBloom® *Nannochloropsis* sp. were formulated for rotifers cultivation since this species demonstrates high rotifers production and is a highly demanded product for their cultivation due to liquid concentrates easy application. Mitigation of the incidence of pathologies was addressed through two different strategies, namely the use of probiotics (VitaBloom®) or reduction of product bacterial load (PhytoBloom® premium products). Probiotics are known to inhibit *Vibrionaceae*, which include several of the most prevalent pathogens responsible for mortalities, and have beneficial effects on the biological performance of the organisms and higher resistance to stress[1]. The reduction bacterial load of the products improves the water quality and avoids the proliferation of potential pathogenic bacteria[2], and is particularly useful for the cultivation of species highly susceptible to the presence of bacteria in the water.

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The reduction of microalgae agglomeration was tackled in all products through the optimization of the formulations processing technologies to reduce sedimentation and the formation of microalgae agglomerates in the water, which is a major constraint since it promotes product sedimentation, debris formation, reduction of water quality and the organism's biological performance. In liquid concentrates, the ionic composition of salt solutions and their salinity was optimized to reduce microalgae agglomeration since *Nannochloropsis* sp. cell wall has a negative charge that influences the interaction with other molecules and particles[3]. Thus, the ionic strength was modulated through different salt solutions to reduce *Nannochloropsis* sp. agglomeration in the water column and improve the product suspension in water. In Rotifers+ an ammonia chelating water conditioner was used in order to reduce the concentration of this metabolite that is toxic to rotifers[4], as it is responsible for inhibiting reproduction and crashing the rotifer population, particularly in the challenging semi-continuous production. The reduction of the number and size of the particles in the water column can promote the reduction of the microalgae sedimentation, thus improving particle suspension on the water column, increasing feed availability of microalgae for rotifers nutrition, reduces waste formation, bacteria proliferation, oxygen consumption and toxic ammonia accumulation in the tanks.

In *A. regius*, Green water product increased 36% larvae survival post transport stress challenge and reduced 108% malformations affecting phenotype compared to green water technique with live *Nannochloropsis* sp. Rotifers+ increased 64% rotifers production in batch and 92% rotifers production in semi-continuous production. Enrich showed 20% more in *S. aurata* survival post-weaning, adequate biochemical profile of enriched rotifers and artemia for *S. aurata* nutritional requirements and a reduction of stress enzymes post transport stress challenge. ELITEf allowed to increase batch rotifers production by at least more 50% than a commercial product and RASf enhanced rotifers production in RotiRAS system at least 50% compared to a commercial product. Moreover, RASf reduced the maintenance of the system since it does not promote the filters clogging of the system and have a reduced debris accumulation in the tanks.

In conclusion, the appropriate design of the formulas of specialized microalgae products made it possible to respect the technical needs of the organisms and aquaculture systems, reducing the effort of human resources, simplifying the daily procedures in the nursery, obtaining higher biological performance of the organisms and a high cost-benefit relation of the product application. All these characteristics are essential to leverage aquaculture profits and sustainable production growth.

**Acknowledgements**
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# EFFECT OF SEASONALITY AND RAFT LOCATION ON THE IMMUNE STATUS AND BYSSUS PRODUCTION OF MUSSELS CULTURED IN GALICIA

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## Introduction

Climate change has been altering the Earth's climatic system, including rising sea temperatures, ocean acidification, extreme weather events, and biodiversity loss[1]. These changes threaten both wild and cultured species[2,3]. *Mytilus galloprovincialis*, one of Europe's most important farmed mollusk species, is primarily cultured on rafts in the nutrient-rich rías—drowned river valleys—of Galicia, on Spain's northwest coast[4]. Due to their filter-feeding lifestyle, mussels constantly face environmental stress and rely on their innate immune system to cope with these challenges. Additionally, mussel attachment to substrate (rocks and culture ropes) depends on the structure of the byssus. Together, immune function and byssus strength are critical for mussel survival. In this study, we investigated how the spatial (raft location) and temporal (seasonality) parameters affect the immune response and byssus production of cultured mussels.

## Materials and Methods

Four rafts were selected in the Ría de Arousa, which hosts 60% of Galicia's mussel rafts: two highly exposed to waves (OuN and OuS), one in sheltered inner areas (InC), and one in the middle section (MidW) with intermediate conditions (Fig. 1A). Six mussels (three at 0-6 m and three at 6-12 m depth) were sampled per raft/season (spring, summer, autumn, winter), then transferred to aquarium facilities for processing (Fig. 1B). To assess the mussels' cellular stress response, the activity of reactive oxygen species (ROS) and nitric oxide (NO) was measured via chemiluminescence and spectrophotometry, respectively. Gene expression related to oxidative stress, immunity, and thermal stress (GST, LYS, HSP70) was also analyzed in gills using qPCR. Byssus production was evaluated through Mgfp3 gene expression to infer the risks of biomass maintenance in the rafts.

## Results and Discussion

Factorial ANOVA revealed that seasonality had the largest effect on all response variables. Raft position ranked as the second most influential factor in the case of NO production and the expression of immune genes. No significant differences were noted for rope depth in any variable. ROS and NO levels were higher in the summer, which appears to be an oxidative stress response to increased metabolism and pathogen pressures resulting from higher temperatures (Fig. 2). The expression of immune genes also varied seasonally, reflecting a targeted seasonal pattern based on food availability, spawning events, pathogen load, and thermal stress (Figs. 3A, B, C). Byssus production peaked in the spring, likely due to favorable conditions for attachment, allowing the mussels to invest more energy in byssus reinforcement to cope with the seasonal changes. It was, however, reduced in summer as mussels probably prioritize stress management (Fig. 3D).

## Conclusions

Despite mussels' range of tolerance, this study highlights the complex relationship between environmental stressors, such as pathogens and weather conditions, and mussel performance. It emphasizes the importance of implementing sustainable aquaculture practices to optimize health and attachment strength while minimizing stress during critical periods. Understanding these dynamics is crucial for adapting mussel farming strategies to the challenges posed by climate change, ensuring the resilience of this vital species.

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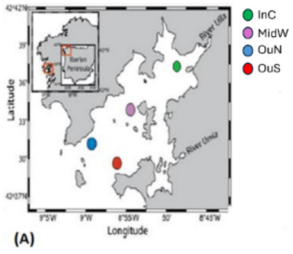


| DATE | SEASON | Nº MUSSELS |
|---|---|---|
| 7/6/2023 | Spring | 24 (1M-24M) |
| 7/9/2023 | Summer | 18 (25M-42M)* |
| 22/11/2023 | Autumn | 24 (43M-66M) |
| 29/2/2024 | Winter | 24 (67M-90M) |

*Sampling not possible at raft 2 (OuS)

(B)

***Figure 1**: **(A)** Map of Ría de Arousa (NW Spain) showing the experimental zones where mussel sampling was conducted. OuN (blue) outer exposed northern site, OuS (red) outer exposed southern site, MidW (purple) middle section of the Ría, InC (green) inner and sheltered site. **(B)** Sampling dates and the total number of individuals sampled per season.*

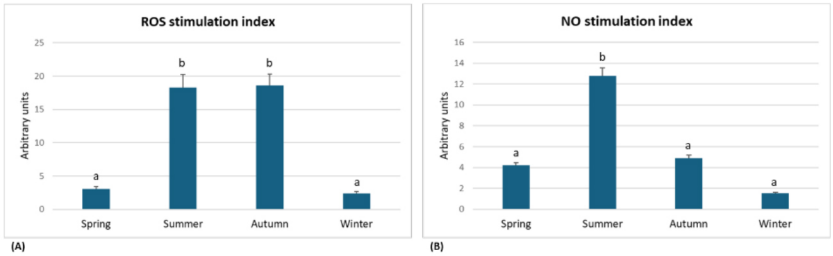


***Figure 2**: Stimulation index of Reactive Oxygen Species (ROS) **(A)** and Nitric Oxide (NO) **(B)** production of mussel hemocytes after stimulation by zymosan, normalized to unstimulated controls (Mean + SEM). Bars indicate the mean of 24 mussels at each sampling season (18 for summer). The same letter indicates non-significant differences ($p > 0.05$).*

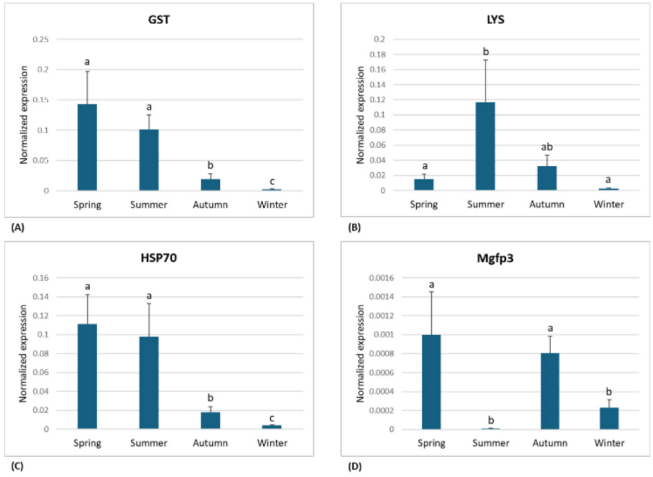


***Figure 3**: Expression of glutathione S transferase (GST) **(A)**, lysozyme (LYS) **(B)**, and heat Shock protein 70 (HSP70) **(C)** genes in mussels' gill and foot protein 3 gene (Mgfp3) **(D)** in mussels' foot (Mean + SEM). The bars indicate the mean of 24 mussels at each sampling season (18 for summer). The y-axis represents the relative expression of each gene normalized to the housekeeping gene (18S). The same letter indicates non-significant differences ($p > 0.05$).*

# MOLECULAR RESPONSE OF THE MEDITERRANEAN MUSSEL TO CONTAMINANT GRADIENTS IN THE GALICIAN COASTAL RÍAS

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## Introduction

The Galician coastline is characterized by a system of drowned river valleys, known as rías, which benefit from nutrient-rich upwelling waters and support extensive aquaculture activities[1]. The Mediterranean mussel, *Mytilus galloprovincialis*, is the most widely cultivated mollusk in Galicia, with a 2022 production of ~200,000 tons, valued at €164 million (FAO)[2]. This makes mussel aquaculture a vital socioeconomic activity in Galicia. However, many of these coastal areas are located near densely populated zones, receiving significant anthropogenic pressure. To ensure the sustainability of this sector, it is essential to monitor the impact of emerging contaminants and pathogens that may affect both human health and aquaculture productivity. This study integrates field sampling and laboratory analyses to assess the molecular responses of mussels to environmental stressors.

## Materials and Methods

Seasonal mussel sampling (winter, spring, summer, autumn) was conducted in 2022/23 at three Ría de Vigo sites along a contamination gradient: Samil (low), Teis (moderate), and Cangas (high). RNA was extracted from the gills and digestive glands of 10 individuals per site and season. Gene expression of stress- and immune-related markers (GST, GPX, CAT, HSP70, C1q, and LYS) was analyzed using quantitative PCR (qPCR). Histological evaluation was performed using paraffin embedding and hematoxylin-eosin staining. Additionally, 1 L of seawater per site and season was filtered (0.22 $\mu$m) and analyzed by specific nested PCR C-D for the detection of Ostreid herpesvirus 1 microvariant (OsHV-1 $\mu$Var)[3].

## Results and Discussion

Gene expression analysis revealed site-specific patterns in gill tissue (Fig. 1A). Mussels from Teis exhibited the highest expression levels for HSP70 (53.96%), GPX (48.45%), and GST (43.65%), suggesting a strong oxidative stress response. In contrast, Samil showed a marked increase in LYS (65.42%), potentially reflecting localized immune activation. Seasonal variation in gene expression was also evident (Fig. 1B). Mussels sampled in summer and autumn exhibited elevated levels of HSP70, GST, and GPX, indicating increased environmental stress during warmer months. In contrast, winter samples showed consistently lower expression across all genes, while spring samples revealed moderate activation, suggesting transitional physiological responses. Gene expression in the digestive gland also varied significantly among sites (Fig. 1C). Mussels from Samil exhibited the highest expression levels for most of the genes, including GST (71.20%), GPX (67.71%), and LYS (62.38%), indicating a robust oxidative stress response and immune activation in this location. Conversely, mussels from Teis exhibited elevated levels of C1q (53.07%) and HSP70 (28.70%), suggesting that different stressors or environmental conditions influenced these markers. Seasonal variation in gene expression within the digestive gland also exhibited distinct patterns (Fig. 1D), mirroring those in the gills. Although histological analyses are still in progress, preliminary findings suggest that there are no detectable tissue alterations across sampling sites or seasons. OsHV-1 $\mu$Var (Fig. 2) was most frequently detected in Cangas during autumn, showing the highest viral load. Additional detections occurred in Teis and Samil during summer, in Cangas during winter and spring, and in Samil during spring. No viral DNA was detected in Teis during autumn and winter, nor in Samil during winter. These results suggest a stronger and more persistent viral presence in the most contaminated area (Cangas), with sporadic detection in moderately (Teis) and minimally (Samil) impacted sites.

## Conclusions

This study demonstrates the effectiveness of integrating molecular, histological, and environmental virological tools to assess mussel health across different contamination scenarios. The strong seasonal and spatial variability in gene expression suggests that mussels are sensitive bioindicators of environmental quality. The detection of OsHV-1 $\mu$Var in multiple locations and seasons highlights the circulation of this pathogen in Galician waters, which may pose a latent threat to aquaculture operations. The qPCR panel used proved to be a reliable tool for early detection of sublethal stress responses. When combined with histopathology and environmental pathogen screening, this approach can support the implementation of monitoring programs in bivalve production areas.

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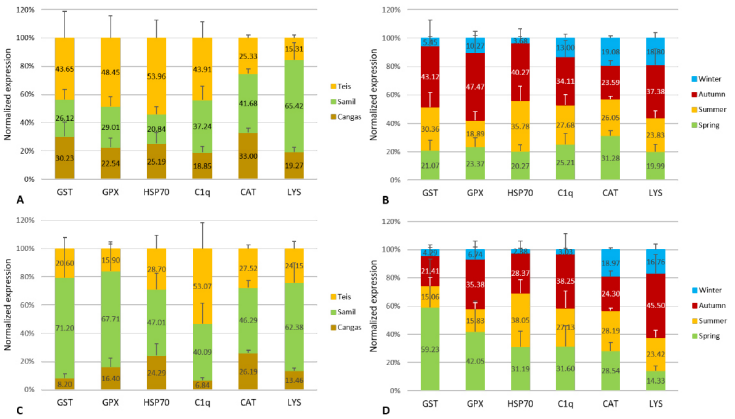


***Figure 1**: Expression levels (Mean + SEM) of six stress- and immune-related genes in gills across three sampling sites **(A)**, four seasons **(B)** and in the digestive gland across the same spatial and temporal gradients **(C, D)**. Bars represent the mean of 10 individuals in 4 seasons per site (A, C) and the mean of 10 individuals in 3 sites per season (B, D).*

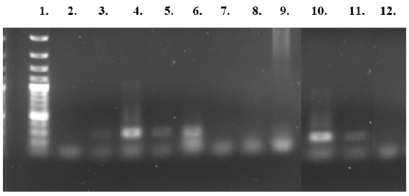


***Figure 2**: Specific Nested PCR for the detection of OsHV-1 µVar. 1. Molecular weight marker, 2. Blank, 3. Cangas-spring, 4. Cangas-autumn, 5. Cangas-winter, 6. Teis-summer, 7. Teis-autumn, 8. Teis-winter, 9. Cangas-summer, 10. Samil-summer, 11. Samil-spring, 12. Samil-winter.*

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# CONSUMERS PERCEPTION TOWARDS CIRCULAR STRATEGIES IN AQUAFEEDS

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## Introduction and background

Aquaculture is pivotal in ensuring food security and nutrient supply to the world's growing population.

The developments of the past decades, which led to farmed production surpassing capture fisheries in 2022 (SOFIA, 2024), have brought to light several challenges related to the sector's sustainable development.

Among the most discussed issues is the need for diets that reduce dependency on raw materials directly "extracted" from nature, such as fishmeal and fish oil. Although plant-based ingredients have helped lower the trophic level of farmed species (Cottrell et al., 2021), adverse effects on fish health and growth, mainly due to anti-nutritional factors and nutrient imbalances, have been demonstrated (Krogdahl et al., 2010). Furthermore, their use has intensified competition for resources needed for direct human consumption and land and water usage (van Riel et al., 2023).

In the European context, where the Green Deal pursuits the ambitious goal of a climate-neutral continent by 2050, and specifically its core "Farm to Fork" strategy, which promotes a healthy, resilient, and transparent agri-food system, have significantly influenced current developments in the livestock and aquaculture sectors. In this regard, circular solutions such as the use of Processed Animal Proteins (PAPs) have attracted considerable interest, as they provide nutrient-rich ingredients, recover raw materials, and offer a local source of protein for animal feed, thereby reducing dependency on imported nutritional sources.

Consumers, who often lack detailed knowledge regarding the feeding practices of farmed animals (Popoff et al., 2017), play a crucial role in adopting these practices, as they are the end users of food products that embody all upstream decisions made along the supply chain. While recent studies on consumer acceptance of PAPs in aquafeeds have mainly focused on insect-derived sources, limited attention has been given to those obtained from poultry and pigs. Against this backdrop, the present study aims to investigate the variables and perceptions that influence the acceptance of such practices among consumers residing in Italy.

## Methodology

The survey was conducted through a questionnaire administered using the CAWI method (Computer-Assisted Web Interviewing).

The questionnaire, divided into different sections and developed based on previous studies on the topic, was designed to qualitatively and quantitatively identify the factors influencing the perception and potential consumption of fish fed with PAPs derived from insects, poultry, and pigs. The included questions were selected to assess sociodemographic characteristics, subjective and objective knowledge, and consumption habits.

One of the strengths of this study is the use of a significant number of respondents, selected through stratified sampling based on population and gender, according to data provided by the Italian National Institute of Statistics. This approach aims to generalize findings to the broader national context regarding consumer perception.

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## Results

The results of this research will be compared with the existing literature, specifically with the findings from a previous study conducted by the authors (Dolfi et al., 2025 in press).

While Italian consumers have generally shown a low willingness to pay for fish fed with insect meal, the present study aims to compare different diets composition to understand consumer perceptions toward different circular practices.

Moreover, given the limited interest previously reported by consumers regarding sustainable farming practices, this study will also provide detailed insights into the reasons behind this attitude.

## Acknowledgements

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# OYSTERS, A SUSTAINABLE BLUEFOOD?

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**Introduction**
Agriculture and terrestrial livestock have long been key sources of protein, but they are also associated with significant environmental impacts, including nutrient pollution and greenhouse gas emissions (Ilea, 2009). In contrast, bivalve aquaculture offers a more sustainable protein production system, requiring no feed inputs and delivering valuable ecosystem services such as nutrient remediation and carbon sequestration (Higgins et al., 2011 and Van der Schatte et al., 2020). This study aims to evaluate the environmental performance of Irish Pacific oyster farming by combining life cycle assessment (LCA) with ecosystem service analysis to understand better its role in reducing eutrophication and mitigating climate change.

**Materials and methods**
This study assessed ecosystem services and environmental impacts of Pacific oyster farming in Ireland using both field sampling and LCA methodology. Morphological and elemental analyses were undertaken on oysters from two production sites, measuring carbon (C), nitrogen (N), and phosphorus (P) content in both tissue and shell. These values were extrapolated to farm and national levels to estimate annual nutrient removal and assign economic value to this ecosystem service. LCA was performed on three sites using primary farm data and secondary databases, covering activities from seed procurement to processing. Environmental impacts were quantified using the CML method, and nutrient and carbon sequestration in oyster shells were integrated into the LCA results to calculate net eutrophication and greenhouse gas emissions.

**Results**
The study found that Pacific oyster farming in Ireland provides significant ecosystem services, removing an estimated 834.3 tonnes of carbon, 33.9 tonnes of nitrogen, and 3.9 tonnes of phosphorus from coastal waters annually. The LCA results showed a carbon footprint of 373.86 kg $CO_2$-eq per tonne of oysters, primarily driven by electricity use and diesel combustion. On the other hand, results showed a eutrophication potential and acidification potential of 0.39 kg $PO_4$ eq. tonne$^{-1}$ and 1.33 kg $SO_2$ eq. tonne$^{-1}$, respectively. Using ecosystem services metrics, one tonne of harvested oysters can remove, on average, 3.05 kg of nitrogen, 0.35 kg of phosphorus, and sequester 70.52 kg of carbon from the environment, thus potentially acting as a nutrient remediator and a short-term carbon sink. From an economic perspective, the valorisation of the nutrient removal potential resulted in an additional income of €1.9 million per year to the Pacific oyster industry, of which N removal accounts for 97.67%.

**Discussion**
This study provides a comprehensive evaluation of Ireland's Pacific oyster farming sector, focusing on its contributions to ecosystem services and environmental impacts through a life cycle assessment. While oyster farming demonstrated lower environmental impacts than other protein production systems (Aubin et al., 2009; Nguyen et al., 2010; Djekic et al., 2015; Kalhor et al., 2016), challenges remain in assessing nutrient and carbon fluxes due to methodological limitations. The study highlighted the role of biodeposition and denitrification in nutrient cycling (Ayvazian et al., 2021) and stressed the need for improved LCA models to capture these services more accurately. Extrapolations were made under justified assumptions, though future research is needed to account for regional variability. Economically, the Irish oyster industry could benefit from nutrient credit schemes and opportunities within the circular economy, such as reusing shells in agriculture and construction. The findings support the integration of oyster farming into environmental management strategies, including carbon farming and water quality improvement, and advocate for the expansion of environmental footprint assessments to inform sustainable aquaculture policy and practice.

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# TEMPORAL DYNAMICS AND CROSS INTERACTIONS BETWEEN WATER AND SKIN MICROBIOMES IN FARMED GILTHEAD SEABREAM

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## Introduction

Fish closely interact with their environment and the microorganisms that inhabit it. Accordingly, increasing attention is being given to the interplay between microbiota, host, and environment. While most studies focus on intestinal bacteria, other microbial niches—such as the skin and the surrounding water—are gaining importance as less or non-invasive biomarkers for monitoring fish health and welfare. There is now evidence that certain behavioural patterns can be modulated by targeting the gut microbiota (Olorocisimo et al., 2023). The relationship between the skin microbiota and specific behaviours has been less studied, although correlation analyses have also supported an association between skin microbiota and reactive or proactive behaviour in gilthead sea bream (*Sparus aurata*) reared at high stocking densities (Toxqui-Rodriguez et al., 2024). To further explore this line of research, the present study aims to characterize how seawater microbial dynamics influence and interact with the skin and rearing water microbiomes throughout the production cycle of gilthead sea bream. Moreover, by understanding the interactions among these three interconnected microbiomes, we explore the potential of using rearing water microbiota as a non-invasive proxy for monitoring host-associated microbial shifts in fish fed different diets formulations.

## Methods

Gilthead seabream juveniles were reared under natural photoperiod and temperature conditions (40°5'N; 0°10'E) at IATS facilities in an open-flow system. During the trial, fish were fed with a CTRL fish meal-based diet, or alternative feeds with processed animal protein (PAP), or insect meal + bacterial protein (ALT) as main novel fish meal replacers. Fish skin and rearing tank water microbiome were sampled at four time points: t0-May (13.2g); t1-July (70.3g); t2-November (295.9g); and t3-February (357.5g). At each sampling point, 9-12 fish per diet were sampled for skin mucus, and water samples were filtered (0.22 µm) to collect bacterial biomass. In parallel, to study seasonal fluctuations in the inlet seawater of the system, water samples were collected monthly during a two-year period from the inlet pipe (Jan-2023 to Dec-2024). After DNA extraction, full-length 16S rRNA (V1–V9) was amplified and sequenced using Oxford Nanopore (PromethION P2). Reads were basecalled (Dorado v0.9, SUP), and taxonomic assignment was performed with minimap2 using SILVA v138.2 as reference database.

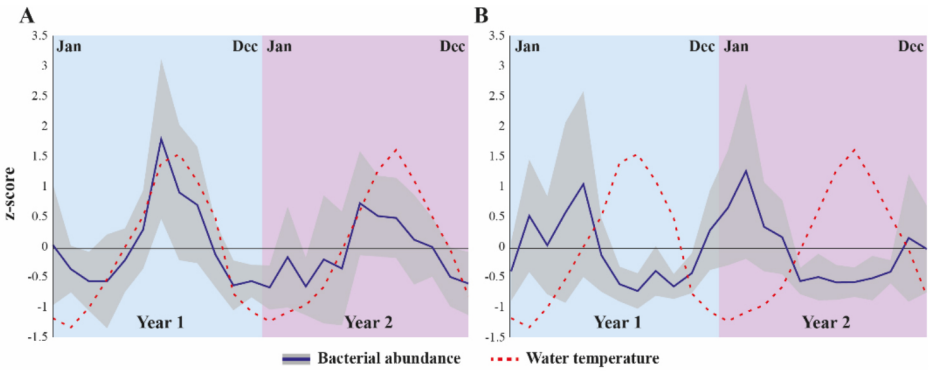


**Figure 1**. Bacterial abundance distribution of bacterial taxa with 12-month period with their peaks of abundance in winter (A) and summer seasons (B).

*(Continued on next page)*

**Results and discussion**

Microbiome composition of inlet and rearing water showed a dominance of three main phyla: Pseudomonadota, Bacteroidota, and Cyanobacteria. Similarly, the skin microbiota maintains the dominance of the first two phyla, while the presence of Cyanobacteria was significantly lower, being replaced by the Bacillota phylum. In terms of OTU prevalence, 130 taxa at genus level were shared consistently between water and the skin mucus. Interestingly, the abundances of 94 taxa (approximately 70% of the total shared microbiota) were positively or negatively correlated between skin and rearing water microbiomes. To refine this set of bacteria selected across time, we applied a time series analysis (RAIN analysis; Coenen et al., 2020) to seawater inlet samples collected across two consecutive years. As a result, among a list of 102 genera ($p < 0.01$) whose abundances were positively (Figure 1A) or negatively (Figure 1B) correlated with seawater temperature, 24 taxa exhibited the same seasonal variation in the skin mucus and rearing water microbiomes. These bacteria (representing 21.1 and 16.8% of the total water and skin abundance) are relevant because apart of being consistent in our facilities environment, can be measured in the rearing water as a non-invasive proxy of a host-associate microbiome.

In addition to time, beta-diversity analysis of skin microbiome revealed that diet represented the secondary source of variation. In fact, even with lower influence compared to temporal variable, LEfSe analysis identified 11 bacterial biomarkers associated with the diet including *Alteromonas, Cobetia* and various members of Paracoccaceae family. Notably, *Alteromonas*—the most abundant diet-discriminant genus—has previously been associated with a reactive behaviour in fish under high-stocking densities (Toxqui-Rodriguez et al., 2024). Interestingly, the ALT diet, enriched in this genus, has also been associated with this behavioural trait, reinforcing a possible link between diet, behaviour, and the skin microbiome.

**Concluding remarks**

This work reveals the cross-talk between the microbiomes of rearing water and fish skin mucus, supporting the potential use of rearing water microbiota as a practical, non-invasive tool for monitoring host-associated microbial shifts. These results reinforce the idea that water microbiome analysis can complement fish health monitoring strategies, offering a non-invasive approach to track broad microbial fluctuations within aquaculture environments.

**Funding**

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# USE OF CALCIFEDIOL IN GILTHEAD SEABREAM (*Sparus aurata*): EFFECTS ON GROWTH PERFORMANCE, BIOCHEMICAL COMPOSITION, SKELETAL ANOMALIES AND HISTOLOGY

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**Introduction**
The study explores the use of calcifediol, a metabolite of vitamin $D_3$, as a dietary supplement in gilthead seabream (*Sparus aurata*). Vitamin D is an essential fat-soluble vitamin that fish are unable to synthesize and so require absorbing it directly from the diet (Lock et al., 2010). While vitamin $D_3$ is commonly used in aquaculture, its metabolite, calcifediol, has shown promise in livestock and rainbow trout (*Oncorhynchus mykiss*) (Atencio et al., 2005; Rider et al., 2023), but remains untested in marine species like seabream. Given the essential role of vitamin D in calcium homeostasis, bone development, and nutrient absorption (Lock et a., 2010; Sivagurunathan et al., 2022), this research aims to evaluate the effects of calcifediol on growth, biochemical composition, skeletal anomalies, and intestinal histology in seabream juveniles.

**Materials and Methods**
A total of 600 seabream juveniles (12.8 ± 1.5 g) were divided into five dietary groups with increasing levels of calcifediol: <2.0 (control), 99.7, 167.4, 298.6, and 825.4 ppb. All diets included 3000 IU/kg of vitamin $D_3$. The fish were reared for 15 weeks under controlled conditions and fed three times daily. Parameters measured included growth performance, feed conversion ratio (FCR), specific growth rate (SGR), biochemical composition, vitamin and mineral content, skeletal anomalies (via X-ray), and intestinal histology among others. Statistical analyses included ANOVA and regression models to determine significance ($p<0.05$).

**Results**
-*Growth Performance*: Fish fed 99.7 ppb calcifediol showed the highest growth and best FCR. Higher doses (298.6 and 825.4 ppb) reduced growth, suggesting potential toxicity.

-*Biochemical Composition*: Whole-body lipid content increased with calcifediol, while protein content decreased. Liver lipid levels rose linearly with supplementation. The whole-body omega 3/ omega 6 ratios describe a higher deposition of DHA and omega 3 in the diet containing 99.7 ppb of calcifediol, whereas the contrary was observed for fish fed the highest level of calcifediol.

-*Vitamin and Mineral Content*: Whole-body vitamin $D_3$ and vertebral calcium increased significantly at 99.7 and 167.4 ppb. Excess supplementation reduced calcium retention.

-*Skeletal Anomalies*: No significant differences were observed among groups.

-*Histology*: Mid-intestine fold length and goblet cell density improved at 99.7–167.4 ppb. Lamina propria width was thinner in supplemented groups, indicating better gut health.

**Discussion**
Calcifediol supplementation at 99.7 ppb significantly enhanced growth, nutrient retention, and intestinal morphology, outperforming traditional vitamin $D_3$. However, higher doses led to reduced growth and calcium retention, indicating a narrow safety margin. The findings align with previous studies showing vitamin $D_3$ toxicity at high levels in seabream (Sivagurunathan et al., 2022). The improved omega-3/omega-6 ratio and DHA deposition at optimal calcifediol levels suggest enhanced lipid metabolism. Histological improvements, such as increased goblet cells and reduced lamina propria width, support better gut health and nutrient absorption.

*(Continued on next page)*

*Table 1. Major findings in the present trial where gilthead seabream werel fed increasing levels of calcifediol during 15-weeks. These include, mean weight, FCR, whole-body fatty acid composition, vertebrae calcium content, mid-intestine fold length, and area occupied by goblet cells per intestinal fold in the anterior intestine (%).*

| | Analysed calcifediol in feed (ppb) | <2.0 | 99.7 | 167.4 | 298.6 | 825.4 | ANOVA (*p* value) |
|---|---|---|---|---|---|---|---|
| Mean weight (g) | day 105 | 75.0±9.5[a] | 80.5±11.9[b] | 77.9±8.6[ab] | 76.7±9.7[a] | 75.4±9.2[a] | 0.000 |
| FCR | 0-105 days | 1.31±0.07[b] | 1.17±0.03[a] | 1.16±0.07[a] | 1.16±0.04[a] | 1.19±0.02[ab] | 0.019 |
| Whole-body Fatty acid composition | OMEGA3/ OMEGA6 | 1.198±0.070[ab] | 1.237±0.011[b] | 1.154±0.070[ab] | 1.178±0.036[ab] | 1.079±0.023[a] | 0.026 |
| Vertebrae | Ca (mg/100g) | 1601±313[ab] | 3123±82[bc] | 3574±704[c] | 2412±505[abc] | 1173±1013[a] | 0.004 |
| Mid-intestine | Fold length | 578±59[ab] | 678±45[b] | 672±29[b] | 626±45[ab] | 550±8[a] | 0.012 |
| Area occupied by goblet cells per intestinal fold (%) | Anterior Intestine | 1.62±0.24[a] | 2.16±0.25[ab] | 2.43±0.15[b] | 2.22±0.15[ab] | 1.87±0.35[ab] | 0.017 |

**Conclusions**

Calcifediol is a potent and efficient vitamin D source for gilthead seabream juveniles. A supplementation level of 99.7 ppb optimizes growth, feed efficiency, vitamin $D_3$ retention, and intestinal health. However, excessive levels (>298.6 ppb) may induce toxicity, reducing performance and mineral retention. These results highlight the importance of precise dosing when using calcifediol in aquaculture diets.

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# EPIDEMIOLOGICAL SURVEILLANCE OF VIRUS OF RISK IN AQUACULTURE: BEYOND KNOWN VIRUSES

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## Introduction

Taking advantage of metagenomics and metatranscriptomics tools, epidemiological studies of human viruses have evolved greatly in recent years. In aquaculture virology, we are a little behind the times, but progress is unstoppable. Epidemiological studies in aquaculture have been carried out for half a century, but the available tools, of the target type, only allowed us to detect those viruses that we already knew about in each geographical area. Nowadays, the availability of this other type of techniques allows us to carry out generic monitoring studies, looking for any type of known and unknown virus present. Preliminary results obtained in a study carried out in Ría de Arousa (NW Spain) show that this technology is useful for understanding the situation of natural and farmed populations, and support the defence of maintaining a permanent system for monitoring the spatio-temporal distribution of viruses in certain marine populations of interest.

## Materials and methods

Samplings were carried out in 5 coastal locations (Fig 1) (4 in Ría de Arousa and one, as a reference, in a nearby estuary), one in an internal area of the estuary, closer to river mouths and towns, 2 in a middle area, with added influence of fish farms, and one closer to open sea. In each case, water (20 L), sediment (1 Kg) and molluscs were sampled at two points, one in an unaffected production area and the other in an area of low or no production. In the present work, preliminary results are shown for sediment and water from two of these areas, one internal and one external.

The viruses were concentrated by reverse capillary osmosis filter systems, followed by ultracentrifugation. For detection of viral RNA sequences, after total RNA extraction and purification, the cDNA molecules library was constructed using a ribosomal depletion preparation kit, and the sequencing carried out in a Illumina Novaseq platform, using 150bp paired-end. For viral DNA sequencing, after extraction and purification of DNA, the library was contructed by the Kapa HyperPrep kit, and sequencing performed in a Illumina Novaseq platform as indicated.

## Results

Although only sequences with E values between 1 $e^{-21}$ and 0, and pairwise identity percentage over 80% were considered, metagenomics led to the discovery of hundreds of contigs with homology to sequences of particular interest in the aquatic environment: algae and microalgae viruses and plankton viruses, e.g., were very abundant. However, as an objective of the project was to determine the presence of viruses that could pose a risk to aquatic animals of interest in aquaculture, Table 1 only presents those viruses that could represent a certain risk to—or at least to have a certain relationship with—aquaculture. We did not observe a clear relationship between presence/absence of risk viruses and the type of coastal area (with or without productivity problems). Instead, we did detect relationship of the degree of virus diversity and the number of contigs for each virus with the location of the areas (clearly higher at the inner sampling points in the estuary).

We have highlighted in the table, using colours, those viruses that are published as posing a proven risk to the species indicated (red boxes) and those for which there is no clear data in this regard (yellow boxes). We also would like to highlight the possible presence of high-risk viruses for molluscs (e.g. pinnea, mussel or octopus) and crustaceans (crabs, prawns and others). Also noteworthy is the large number of contigs (more than 40) of betnodavirus, a virus that poses a risk to fish farming, especially Senegalese sole betanodavirus (31 contigs), which has a proven risk for that species, which is of high interest to fish farming in this area.

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Table 1.- Viral sequences corresponding to aquaculture risk viruses detected by metagenomics in water and sediments sampled in 2 coastal locations at the Arousa estuary.

| **Host** | | **Virus type** | |
|---|---|---|---|
| Bivalve molluscs | Mussels | Rhabdovirus | Chemarfal virus 1 |
| | | Densovirus | Clidnapec virus |
| | Other | Picorna-like | Beihai picorna-like virus strain 32 |
| | | Circovirus | Circovirus-like SAR-B |
| Other | Octopus | Picorna-like | Beihai picorna-like virus strain 32 |
| Crustacea | Crabs | Picorna-like | Beihai picorna-like virus strain 32 |
| | Prawn | Iridovirus | Acetes erythraeus iridovirus |
| | | Nimavirus | White Spot Syndrom Virus |
| | Other | Circovirus | Circovirus-like SAR-B |
| Echinoderm | Sea star spp | Densovirus | Asterias forbesi-assoc. densov. |
| | | Circovirus | Starfish wasting disease |
| Fish | Salmonids | Orthoreovirus | Piscine Orthoreovirus |
| | Senegalese sole | Betanodavirus | Recombinant NNV |
| | Gilthead sea bream | Betanodavirus | Recombinant NNV |
| | Pearl gentian grouper | Betanodavirus | RGNNV |
| | Redspot grouper | Betanodavirus | RGNNV |
| | Tiger grouper | Betanodavirus | RGNNV |

**Discussion and Conclusions**

The preliminary results of this study demonstrate, first, that there could be more viruses posing a risk to aquaculture in the study areas than are currently known in this region and, second, that the potential risk is greater in the innermost areas of the estuary. However, the presence of the sequences detected in metagenomics, although reliable (due to the restrictive criteria used), is not sufficient to demonstrate the presence of those viruses. Therefore, primer/probe sets are currently being selected to apply real-time PCR and digital PCR to demonstrate or rule out their presence.

**Acknowledgements**

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# DEVELOPMENT AND NUTRITIONAL EVALUATION OF AN ARTISANAL CANNED AMAZONIAN SILVER CROAKER (*Plagioscion squamosissimus*): ADDING VALUE TO THE REGIONAL FISH SUPPLY CHAIN

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**Introduction**

Fish is considered a privileged meat product from a nutritional point of view, as it is rich in polyunsaturated fatty acids (especially omega 3), has high-quality protein, digestibility, biological value, and is also a source of vitamins and minerals (Dias and Silva, 2021). In the Amazon region, fishing is considered the basis of the economy of some cities and traditional communities, which stands out for the wealth of fish that are exploited and the quantity of fish and shrimp caught annually.

With this in mind, we considered technologies related to value-added preserves (new cuts, new sauces, new ingredients) developed from fish, intended for human consumption and nutrition. Therefore, fish is the main food source in the Amazon region and according to FAO data indicating that this same consumption tends to increase on a national and global scale, this work aimed to develop an artisanal preserve made from silver croaker (*Plagioscion squamosissimus*) in tomato sauce and to characterize this product proximally.

**Material and methods**

The fish were purchased at a street market in the municipality of Macapá, state of Amapá, Eastern Amazon, through donations. The fish were inspected and then scaled, their heads, viscera and fins removed, and the parts separated for canning.

Then, the parts intended for canning were immersed in hyperchlorinated water in accordance with industrialization and quality control standards. The fish were cleaned, processed (special cuts) and placed in glass jars, and the topping sauce was then added, and the jars were then covered and subjected to the cooking process. The jars containing the ingredients underwent heat treatment (cooking) for 50 minutes, and the pressure reduction should be allowed to drop for 10 minutes before opening the pot, to prevent the lids from bulging. After removing the jars from the pressure cooker, they were allowed to wait until the internal boiling of the glass ended and then they were inverted until they cooled.

After preparing the preserves, moisture, ash, total lipids and total proteins were determined according to the methodologies proposed by official analysis methods (AOAC, 1990), carbohydrates and caloric value as described by Dias and Silva (2021). The centesimal composition analyses were performed by groups of triplicates.

**Table 1.** Proximate composition of artisanal Amazonian silver croaker (*Plagioscion squamosissimus*) in tomato sauce, preserved in a glass jar.

| Parameter | Silver Croaker *(Plagioscion squamosissimus)* |
|---|---|
| **Humidity (g/100g)** | 82.46 ± 2.02 |
| **Mineral waste (g/100g)** | 0.23 ± 0.26 |
| **Total lipids (g/100g)** | 2.32 ± 0.15 |
| **Total proteins (g/100g)** | 12.10 ± 2.25 |
| **Carbohydrates (g/100g)** | 2.74 ±0.78 |
| **Calorific value (Kcal/100g)** | 71.24 ± 0.84 |

*(Continued on next page)*

**Results and discussion**

After comparison with the literature, it was possible to conclude that artisanal canning presented high levels of moisture (82.46±2.02 g/100g), low levels of ash (0.23±0.26 g/100g), lipids (2.32±0.15) and calories (71.24±0.84 Kcal/100g), and moderate levels of protein (12.10±2.25 g/100g) and carbohydrates (2.74±0.78 g/100g).

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The importance of these data for the Amazon is significant, as they highlight the potential of artisanal fish canning as a nutritious and economically viable source of food in the region. The high moisture content contributes to the palatability of the product, while the low ash and lipid contents are indicative of a healthy food with low levels of impurities and fat. Moderate amounts of proteins and carbohydrates ensure nutritional balance, which is essential for the diet of local populations.

According to Gonçalves (2021), the importance of developing and producing fish-based products is not limited to: food safety; maintaining quality; including species that are rarely consumed; adding value to unattractive fish; and, most importantly, encouraging fish consumption. Therefore, fish-based products are among the most important food products traded internationally.

**Conclusion**

These results demonstrate the viability of producing canned fish in the Amazon, using local ingredients and artisanal methods, promoting sustainability and the valorization of regional natural resources. Thus, the dissemination of these data at scientific events is crucial to increase the visibility and recognition of the nutritional and economic value of these products. Thus, the canned fish prepared by this study is easy to prepare and handle, and can be replicated at low cost, since materials and ingredients that can be easily found in markets and fairs are used. The canned fish has been shown to have a good dietary standard, as it has nutritional components that can be used in diets that require proteins and lipids, which nutritionally justifies encouraging the consumption of these artisanal canned fish.

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GONÇALVES, A. Tecnologia do Pescado: Ciência, Tecnologia, Inovação e Legislação. São Paulo: Atheneu, 540p. 2021.

# PHOSPHORUS REQUIREMENTS IN SEA-CAGE FARMED ATLANTIC SALMON WITH AN EMPHASIS ON BONE HEALTH AND DIGESTIBILITY

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**The role of phosphorus in salmon aquaculture**

Phosphorus is an essential nutrient for Atlantic salmon (*Salmo salar*, L.) due to its role in cellular metabolism and bone mineralisation. As the level of phosphorus in water is low and its form in readily available ingredients is often poorly digested, commercial fish feeds are supplemented with highly digestible inorganic phosphorus, a limited and expensive resource. It is therefore essential that salmon are fed only the necessary amounts to not only reduce costs, but to prevent the release of excess phosphorus into the environment. The required dietary phosphorus levels during the grow-out phase of farmed salmon, which is when feeding intensity is highest, remain to be specified. The range of total phosphorus in commercial diets can be relatively high (9.4–3.0 g/kg total P) (Chatvijitkul et al., 2017) compared to the recommendations (8.0–10.0 g/kg total P) (National Research Council, 2011). The Norwegian annual production of Atlantic salmon was 1.5 Mt in 2021 while the average phosphorus emissions from salmon farming reached 16.3 kt (Pandit et al., 2023). With the salmon industry expected to reach 5 Mt annually by 2050, it is essential to ensure proper phosphorus management.

**Experimental design**

In the current study, sea-cage reared salmon were located at the MOWI Feed Research Station (63°N, 7°E) from December 2022 (1.8 kg), through April (2.8 kg), until July 2023 (4.2 kg). The temperature was 5–9 °C (December-April) and 7–14 °C (April–July). Salmon were fed one of six diets (A–F) formulated to contain a total phosphorus level of either below (Diets A–D, 6.1, 8.0, 8.7, and 9.5 g/kg, respectively), at (Diet E, 10.4 g/kg), or above (Diet F, 11.2 g/kg) the current recommendations. The study involved 24 sea-cages, with 4 replicates per diet and 90 fish per sea-cage. Diets with 57.4% plant protein and 7.5% fish meal were supplemented with mono-ammonium phosphate (MAP) (Diets B–F). The MAP inclusion rate in Diet A was negligible and raw materials were the main source of dietary phosphorus. Response parameters were growth, phosphorus digestibility, phosphorus excretion and several skeletal health indicators, namely bone mineralisation and bone deformities since inadequate or excess dietary phosphorus content can play a role in deformity development. In addition, bone mechanical strength measured as resistance of the vertebra to deformation under stress, was analysed as it is a sensitive biomarker for detecting sub-optimal phosphorus levels.

**Growth and bone health**

In all diet groups, growth was slower from December–April compared to later in April–July. From December–April, all diet groups grew at comparable rates of about 0.62 mm/day and 0.35% body mass/day. The general growth rate in animals fed Diets B–F increased between April–July to 0.98 mm/day and 0.55% body mass/day. However, during this latter period, Diet A was not able to sustain the same growth rate with the fish growing significantly slower at 0.77 mm/day and 0.47% body mass/day. According to radiological examination there were no dietary differences in the prevalence of deformities except for the occurrence of increased radiolucent spaces due to large areas of non-mineralised bone in those fish fed Diet A. In April, animals fed Diet A already showed a significant reduction in vertebral ash content and mechanical strength by 20% and 39%, respectively, compared to those fed Diets B–F. In July, vertebrae of animals fed Diet B also exhibited a 7% and 18% reduction in ash and mechanical strength, respectively, compared to animals fed Diets C–F.

**Phosphorus digestibility and excretion**

Phosphorus digestibility was lower in December-April compared to April–July where there was a most notable increase in animals fed Diets B–D. The increased phosphorus digestibility led to an increase in the level of available dietary phosphorus from 2.3, 3.7, 4.1, 4.6, 5.6, and 5.8 g/kg in Diets A–F, respectively, in the period December–April, to 2.5, 4.2, 4.6, 5.1, 5.8, and 6.1 g/kg in April–July.

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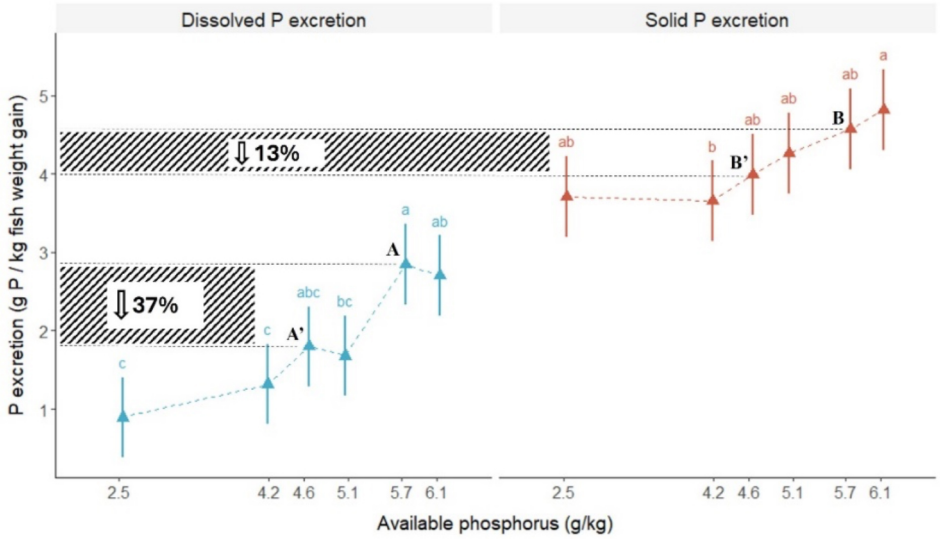


Figure 1. The calculated estimated rate of phosphorus (P) excretion in animals fed different levels of available P. The cross-out areas specify the potential reduction in dissolved and solid P excretion between the currently used P levels in commercial diets (A, B) and the updated requirements based on this study (A', B'). The statistical significance between diet groups is specified with different lowercase letters.

## Conclusions

The levels of available phosphorus required for regular bone mineralisation and mechanical strength were 3.7 g/kg (Diet B) in the period December–April and 4.6 g/kg (Diet C) in April–July. Therefore, there is potential to reduce the total dietary phosphorus content in current commercial feeds, here represented by Diet E (10.4 g/kg), by 16–24%. This will not only reduce feed costs, but may also significantly lower the total phosphorus loss into the environment by estimated 13% reduction in solid phosphorus excretion and 37% reduction in dissolved phosphorus excretion (Fig. 1).

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# ADVANCES IN THE PROOF OF CONCEPT OF A FEED RESTRICTION STRATEGY FOR IMPROVED SUSTAINABLE PRODUCTIVITY IN ARCTIC CHARR (*Salvelinus alpinus*) CULTIVATION: BANKING ON LIPID METABOLISM DYNAMICS TO GENERATE FULL CATCH-UP GROWTH

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The charr (*Salvelinus sp*.) genus is native to the Holarctic realm and displays adaptative metabolic mechanisms that rely on lipids to sustain energy homeostasis through prolonged overwinter feed scarcity and short nutrient rich spring-summer cycles. Arctic charr (S. alpinus), the most starvation-adapted species exhibits outstanding coping mechanisms that stem from dynamics in lipid class composition, storage-to-mobilization ratio, and endocrine regulation when fat storage tissues are depleted. This metabolic cascade can result in body mass loss that can be compensated for via the triggering of an accelerated growth response associated with improved feed conversion rates upon refeeding. Accordingly, feed restriction (FR) as a production strategy in Arctic charr aquaculture is increasingly studied to reduce costly feeding operations and phosphorous discharge provided, 1) fish can withstand substantial FR without jeopardizing growth, or 2) complete compensatory growth (CG) is successfully expressed following severe FR. In the past decade, our team has tested various FR models applied at commercial-scale within production cycles of partner local fish farmers and was able to adapt the approach based on environmental parameters, farm site, species and growing-out to distribution timeframes. We recently integrated notions of physiological phases of starvation (Phase I: Glycogenolysis; Phase II: lipolysis to fatty acid oxidation; Phase III: Protein catabolism) for the development of adapted FR protocols essentially based on the predispositions of Arctic charr to delay the transition to protein catabolism (Phase III), thus extending feed restriction-associated productivity benefits. Recent experimental results indicate a whole carcass lipid depletion threshold target of 18% in sub-adults (180g) prior to refeeding is associated with the fastest compensatory growth response ever reported in the literature. Standard lipid content determination techniques provided *a posteriori* values, required lethal techniques and costly analytical protocols. In a most recent trial conducted on adult fish (>400g), we tested a non-invasive approach to gauge lipid depletion *in continuo* during a FR sequence using microstrip sensor technology. The strategy successfully identified a >18% depletion that signaled the perfect refeeding timing in early spring that triggered a complete CG response, unveiling scalable sustainable profitability.

# TOWARDS CIRCULAR BIOECONOMY: NUTRITIONAL AND BIOCHEMICAL CHARACTERIZATION OF SEA LETTUCE (*Ulva* SP.) CULTIVATED IN ASSOCIATION WITH A FISH FARM

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Fish aquaculture produces a nutrient-rich water that may impact the surrounding ecosystems if discharged. Integrating aquaculture with macroalgae cultivation could provide a sustainable solution by simultaneously cleaning this water, producing valuable biomass and boosting circular bioeconomy. *Ulva* sp., a fast-growing green seaweed, has high potential for bioremediation, as it efficiently absorbs excess nutrients from aquaculture waters (Bolton et al. (2016), Shpigel et. (2019), Duarte et al. (2025)). Additionally, *Ulva* species have a rich and diverse composition, containing significant levels of proteins, fibers, sulfated polysaccharides such as ulvan, pigments, vitamins, and minerals (Bolton et al. (2016), Hofmann et al. (2024)). However, the values of these nutritional and biochemical compounds can vary according to the season and the culture conditions, including stocking density and flow rate (Hofmann et al. (2024)).

The objective of our study was to perform a characterization of the nutritional and biochemical properties of *Ulva* sp., which was cultivated under three stocking density levels (D1, D2 and D3) and two flow rate levels (low–F1 and high–F2) in the water from a fish farm for one year. Every month, the nutritional and biochemical composition was assessed in term of protein, total polysaccharide, ash, carbon, nitrogen, pigment (chlorophyll *a*, *b* and total carotenoid), total phenolic content and antioxidant capacity. A $Z_{score}$ was performed for each variable across cultivation conditions and seasons to assess the best performance.

Results showed that *Ulva* sp. composition varied over the year and across the stocking density and flow rate. Protein (17.2-30.2%), polysaccharide (9.6-41.4 %), ash (12.9-25.8 %), carbon (25.1-36.5%) and nitrogen (3.4-6.0%) content, total pigments (3.6-11.1 mg g $DW^{-1}$), phenols (0.6-2.6 mg /g DW) and antioxidant capacity (14-86 mg TE/ g DW).

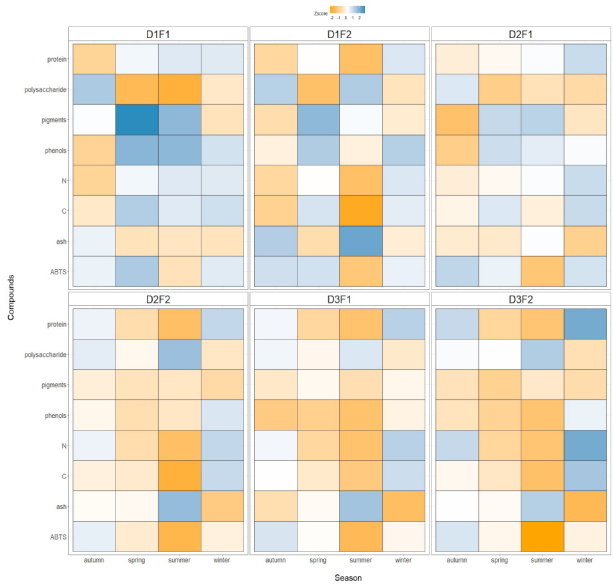


Figure 1. Heatmap of the *Ulva* sp. composition contents (Z-scored) over the season and the cultivation conditions.

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Cultivating *Ulva* sp. at D1F1 showed an overall high performance (blue color dominance, Fig.1), in terms of pigment, phenol, nitrogen, carbon and protein content, especially in spring/summer. Additionally, this cultivation condition showed high productivity in terms of growth (data not shown). This work will explore the influence of the cultivation conditions and the seasonal effect on this nutritional and biochemical composition of *Ulva* sp.

Acknowledgments
The authors are thankful to Aquacria Piscícolas S.A. the opportunity to collaborate on this study. This work was financially supported by "Pacto da Bioeconomia Azul" (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line "Agendas for Business Innovation" through the funding scheme C5 - Capitalization and Business Innovation, by the Portuguese Foundation for Science and Technology (FCT) with a PhD scholarship (UI/BD/150932/2021) under the program BYT PhD CIIMAR and by the project SITE (ref. FA_05_2017_010) funded by Fundo Azul.

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# BLUEBOOST - CULTURE OF A WIDE RANGE OF LOW TROPHIC SPECIES TO BOOST SUSTAINABLE PRODUCTION OF BLUE FOOD AND REDUCE ENVIRONMENTAL FOOTPRINT

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## Introduction

The EU is the world's largest importer of seafood (3,500 M€) with a self-sufficiency rate of only 27.8% in 2023 while EU seafood demand is increasing. The EU with its strategic guidelines for a more sustainable and competitive aquaculture sector, as well as strategies for the development of the blue bioeconomy, has clear ambitions to increase production from sustainable aquaculture. An integral part of this strategy is the enhancement of low trophic species production and the introduction of novel farming methods able to sustainably intensify aquaculture production, such as the development of Integrated Multi-Trophic Aquaculture (IMTA) systems. However, the uptake of IMTA and low trophic species culture by the industry has been disappointing due to significant challenges linked to systems complexity and the dominance of economic considerations in the decision-making process. However, low trophic species production can be integrated with higher trophic fed aquaculture species in a way that the wastes from the higher trophic species become resources for lower trophic levels using IMTA systems. Importantly, the strong but narrow and static economic focus on private costs and benefits that has so far limited IMTA uptake neglects environmental externalities and associated societal costs and benefits, such as utilisation of wastes at present causing eutrophication and stimulation of production circularity and pathways towards zero-carbon emissions. The present state of the art acknowledges these possible environmental benefits of low trophic aquaculture and IMTA, but it does not quantify nor value those benefits, nor the societal gains from increased resilience. The challenge is to generate this data and show stakeholders the environmental and economic benefits of low trophic species culture and IMTA.

BLUEBOOST aims to address these challenges:

a) Increase the aquaculture industry´s uptake of technologies for the culture of low trophic species together with established fed species to diversify and increase aquaculture production.
b) Describe and quantify the environmental and economic benefits of the culture of a wide range of low trophic species together with established fed species for stakeholders, in the aquaculture industry, policymakers and consumers.

## Materials and Methods

BLUEBOOST partners have set up six IMTA case studies integrating the culture of low-trophic species (omnivorous fish, crayfish, molluscs, sea cucumber, sea urchin, seaweed, oysters and plants) with established aquaculture species (fish and shrimp) in Spain, Portugal, Italy, Poland and Brazil. In order to objectively evaluate, quantify, compare and adaptively reduce the environmental impacts of aquaculture with the implementation of IMTA production systems, Life Cycle Assessment (LCA) is used to assess the environmental impact (e.g. use of energy, land, water and greenhouse gas or nutrient emissions) of products and supply chains, a method underlying the EU Product Environmental Footprint (PEF) standard. The economic viability of these novel systems will also be assessed as profitability is a prerequisite for wider uptake and has been an obstacle for the development of IMTA systems to date. BLUEBOOST is an impact driven project that has applied the methodology of the Theory of Change to define pathways that show how project outputs can be transformed through communication to outcomes and societal change.

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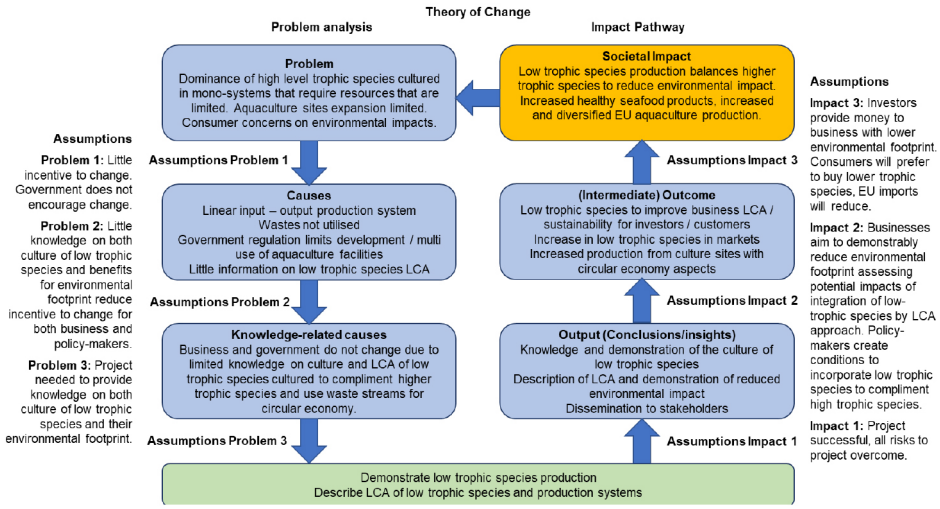


## Results and Discussion

At the heart of the required changes is the industries' ambition to sustainably intensify production activities and maximise economic return, together with society's legitimate expectations to see the environmental footprint of goods and services being reduced. BLUEBOOST proposes to accelerate change in the aquaculture industry by addressing significant knowledge gaps that exist in the conceptual development, practical implementation and regulation of integrated aquaculture systems (Problem analysis) to provide outcomes and a clear pathway to change. BLUEBOOST outcomes predict the aquaculture industry will improve environmental credentials of businesses and products, consumers will demand low-carbon impact products and business investments and policymakers will provide a positive environment for the co-culture of low trophic and established species and deploy regulation to capture waste and reduce pollution. Therefore, Government policy and changing expectations of investors and consumers will drive aquaculture transition to carbon neutrality as shown in the impact pathway in the Theory of Change figure. This win-win scenario will deliver improved diversification of blue food / feed products and, at the same time, will increase production volumes to improve business economic returns combined with a reduced environmental footprint. Therefore, BLUEBOOST can deliver a concrete roadmap for the aquaculture industry to demonstrably reduce its environmental impact and increase sustainable blue food and feed production that can lead to societal transformation.

This societal transformation would generate changes such as: large increase in production of low tropic species, sustainable carbon neutral aquaculture, less pollution, less eutrophication, more wealth in rural coastal areas, improved costal environment, restored ecosystems, more species diversity and healthier diets.

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# RECOMBINANT GONADOTROPIN INDUCED OUT-OF-SEASON SPAWNING AND LARVAL CULTURE OF FLATHEAD GREY MULLET (*Mugil cephalus*)

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## Introduction

The flathead grey mullet (*Mugil cephalus*) has been recognised as an economically important euryhaline and eurythermal species with potential for aquaculture diversification in the Mediterranean and other regions, such as the Republic of Korea, Taiwan, and South Africa. The species positive attributes for aquaculture include good adaptation to captivity, rapid growth (Whitfield et al., 2012), omnivorous and detritivore feeding habits (Vinatea et al., 2018), and a valued food market including "bottarga" (the roe of fully mature females) with a market value exceeding 200 euros per kilogram. However, a bottleneck for mullet aquaculture development is reproductive control in captivity. Depending on the region and the broodstock holding system, reproductive dysfunctions have been reported to arrest reproductive development. In intensive systems mullet exhibited a severe reproductive dysfunction where the females fail to progress through the vitellogenesis phase and males fail to generate fluid sperm. Studies in IRTA have overcome these severe reproductive dysfunctions with a protocol of weekly injections of recombinant gonadotropins (rGth) (recombinant follicle stimulating hormone – rFsh and luteinizing hormone - rLh) to induce gametogenesis and spawning (Ramos-Júdez et al., 2022). The aim of this study was to use the rGth protocol to induce spawning outside the natural spawning period and further characterise the quality of gametes for larval culture of mullet.

## Material and Methods

The experiment was conducted between March 20th and July 31st, 2023, to induce gametogenesis in mullet using recombinant hormones. The experiment involved 15 mullet randomly selected from a stock of 38 wild adult mullet introduced into IRTA´s facilities (IRTA Aquaculture, Centre de La Rápita, Spain) between 2015 and 2018. Eight females and seven males were divided into an rGths (supplied by www.raraavis-bio.com) group that was treated species specific recombinant follicle stimulating hormone and recombinant luteinizing hormone and a control group treated with saline. The eight females selected for the study had 105.8 ± 5.81 $\mu$m oocyte diameter. The treatments of the females initiated on staggered dates, every two weeks a pair of one rGths female and one control female received the first recombinant hormone and saline respectively. The treatment of each pair commenced on March 20, April 3, April 17, and May 2, 2023. This programmed the spawning of the females to be every two weeks to facilitate live food production for the larval rearing. The rGths (rFsh and rLh) were administered to mullet following the protocol described by Ramos-Júdez et al. (2022). The eggs were incubated under constant conditions, and hatched larvae were transferred to different 100 and 500 L tanks. The larvae were reared with green water (microalgae Tetraselmis Chuii and Isochrysis galbana), rotifers, Artemia and Gemma wean diets (Skretting) based on protocols from Vallainc et al., (2021). Larval growth, development and survival were registered.

## Results & discussion

In the present study, both female and male mullet were induced with rGths to mature and spawn in June – July, a period outside the natural spawning season of wild populations, which typically occurs in September — October in the Western Mediterranean. All four females were during a 13-week period induced from the early stages of pre-vitellogenesis to complete maturation and ovulation. Over a 5-week period, all three rGth treated males developed from having no sperm present to producing and releasing sperm with spermiation sustained for 10 weeks. Three females spawned with males to give fertilised eggs that were incubated and larvae cultured. Juveniles were obtained with a maximum survival of 14.1 ± 5.2% to 42 days post hatch (final experiment day). In contrast, control females and males remained in the early stages of gametogenesis and did not produce viable gametes, highlighting the success of out-of-season induction with rGths. The present study has for the first

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time demonstrated that rGth hormones can be employed to induce gametogenesis, maturation and spawning of a teleost species out-of-season. Egg and larval quality were sufficient to show that, with further refinement, these protocols can be used by the aquaculture industry as a basis for juvenile production. The high efficacy of induced maturation, over 90% observed in the present and previous studies that developed the protocol, indicate that it offers an attractive alternative for breeder management. Therefore, the findings of this study hold crucial implications for aquaculture, as the culture of this species relies heavily on wild juvenile resources, with hatchery-produced juveniles being scarce.

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# IMPROVING PELLET WATER STABILITY IN AQUAFEED PRODUCTION WITH INTACT AQUA

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## Introduction

In aquafeed production, pellet water stability is a crucial quality parameter—especially for species such as shrimp, which consume feed slowly from the pond bottom. Poor pellet stability can result in nutrient leaching, increased feed waste, and deterioration of water quality. Using effective pellet binders can significantly improve feed performance, animal health, and production efficiency.

This study compares a newly developed non-synthetic binder, Intact Aqua, with a conventional urea-formaldehyde-based binder (PMC), banned in many regions of the world, focusing on improvements in/maintaining water stability, the energy use/ processing improvements are secondary benefit.

## Materials and methods

The trial was conducted at a commercial shrimp feed facility in Central America. A standard shrimp mini pellet formulation was used across all treatments. The control diet included 0.22% of a conventional urea-formaldehyde-based binder (PMC), while test diets contained Intact Aqua at 0.30%, 0.50%, and 0.75% inclusion levels. Each treatment was produced in a 5.5 metric ton batch.

## Results

The most notable improvement was observed in pellet water stability: +4% improvement at 0.3% inclusion, +6% improvement at 0.5% inclusion; +16% improvement at 0.75% inclusion (Fig. 1). Even at a similar dosage as the reference we found improvement of the water stability. These results demonstrate that Intact Aqua provides strong binding effect similar or better than PMC, helping pellets remain intact longer in water. This is essential in shrimp farming, where feed often remains in water for extended periods.

Intact Aqua also improved several production parameters: conditioning temperature increased by +0.8°C to +5.9°C, supporting better pellet formation and durability (Fig. 2) Pellet mill amperage decreased by up to 6.2%, reducing energy consumption (Table 1). Production throughout was also increased, pellet mill performance was consistent and stable.

This trial shows that Intact Aqua is an effective and sustainable pellet binder for aquaculture. It clearly improves pellet water stability, which is critical for shrimp and other aquatic species, while also enhancing processing efficiency and reducing energy use. These combined benefits make it a sustainable, high-performing alternative to synthetic binders in the aquafeed industry.

Figures 1. Water stability of pellets from the cooler

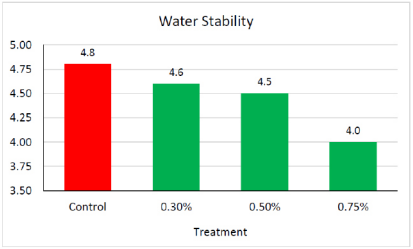


Figure 7. Average conditioning temperature of pelleting runs

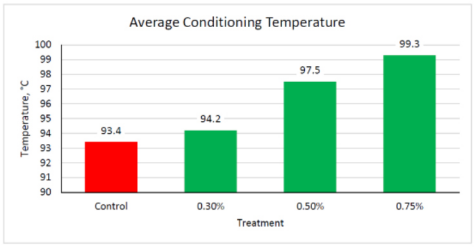


Table 1. Averages of parameters and results of electrical consumption

| Treatment | Average Amps | Average Temperature, °C | kWh/t | %Change | Cost of kWh/ton |
|---|---|---|---|---|---|
| **Control** | 163.08 | 93.4 | 22.16 | N/A | $ 2.22 |
| **0.30%** | 156.85 | 94.2 | 21.31 | -3.82% | $ 2.13 |
| **0.50%** | 152.92 | 97.5 | 20.78 | -6.23% | $ 2.08 |
| **0.75%** | 157.65 | 99.3 | 21.42 | -3.33% | $ 2.14 |

# EUGENOL-DERIVED SELF-ASSEMBLED NANOPARTICLES (E-SAN) AS A FUNCTIONAL FEED IN WHITE SHRIMP *Litopenaeus vannamei*

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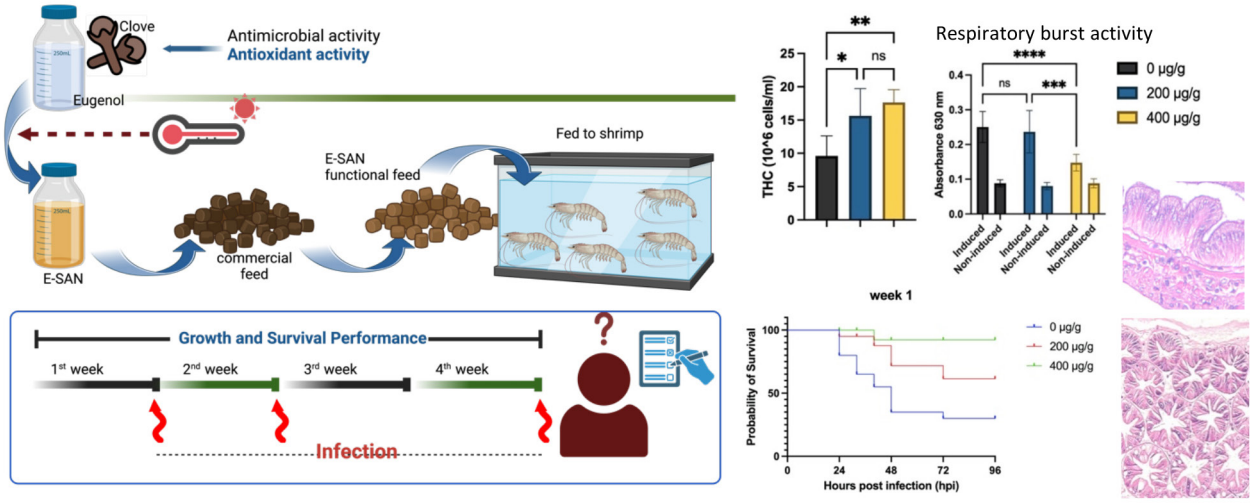
Eugenol-derived self-assembled nanoparticles (E-SAN) offer potential benefits to shrimp immunity due to their antioxidant and antimicrobial properties. This study evaluates the effects of E-SAN on growth performance and shrimp immunity, particularly its protection against Vibrio parahaemolyticus infection (AHPND). The experiment was divided into two groups: one group was subjected to challenge tests, while the other was used to observe growth performance. Twenty shrimp, with an average body weight of 4.6 ± 0.3 g per shrimp, were reared in aquariums (60 × 45 × 40 $cm^3$) containing 96 L of filtered seawater in a circulatory system maintained at a temperature of 28°C. The shrimp were fed commercial pellets supplemented with three different concentrations of E-SAN: 0 µg $g^{-1}$, 200 µg $g^{-1}$, and 400 µg $g^{-1}$. The results revealed that functional feeds containing E-SAN protected the shrimp from AHPND without negatively affecting their growth performance. The protective effects of E-SAN were observed as early as the first week of administration and persisted until the end of the culture period. However, further analysis is needed to optimize the dosage, as histological examination of the hepatopancreas showed structural changes. These findings suggest that E-SAN has great potential as a dietary supplement to enhance shrimp health and resilience against infectious diseases.




**Graphical Abstract**

# SURVIVAL OF EMBRYOS AND FRY OF SEA TROUT (*Salmo trutta M. Trutta*) GROWING FROM EGGS EXPOSED TO DIFFERENT CONCENTRATIONS OF SELENIUM DURING EGG SWELLING

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**Introduction**
Selenium-poor areas are defined in the Earth. Adequate selenium (Se) consumption enhances the health and growth of organisms, but overdose of it can be harmful and pathogenic. The study's objective was to analyse the impact of short-term exposure of sea trout fertilised eggs to inorganic selenium ($SeO_2$) at concentrations from 0 to 32 mg Se $L^{-1}$ to find the optimal and toxic dose of Se on early fish development. Se accumulated in the body, embryos' survival rate, and growth in the first four months of life was examined.

**Materials and methods**
Eggs used in the study were from wild sea trout (*Salmo trutta* m. *trutta* L.) entering the Rega River for spawning (NW Poland). Se content in unfertilised egg and hatchery water was very low (selenium-poor area). In egg, the element concentration was 1.6 μg Se $g^{-1}$ (d.w.) (assuming 65% moisture of eggs) and in aquarium water of 0.04 μg Se $L^{-1}$.

Eight concentrations of selenium 0, 0.5, 1, 2, 4, 8, 16 and 32 mg Se $L^{-1}$ ($SeO_2$) dissolved in aquarium water. Fifty fertilised eggs were placed in 100 mL of selenium solution for 3 hours to swell at 6 °C. After the swelling, the eggs were rinsed with separated aquarium water and transferred to baskets floating in the basins (three repetitions) with a closed water circuit. The survival rate was monitored by counting up to the age of four months after hatching.

**Result**
Accumulation of Se in the body of hatch increased with the increase of element concentration in the aqueous environment during egg swelling. Swelling of fertilised eggs in water supplemented with Se at a concentration from 0.5 to 8 mg Se $L^{-1}$ was associated with a slightly positive impact on the hatching rate. Higher concentrations of Se were harmful to embryo survival, while 32 mg Se $L^{-1}$ was lethal. The selenium content in hatch body in the control group average 3.6 ± 0.2 μg $g^{-1}$ (d.w.), whereas, in the 16 Se group, selenium concentration was 5.4 ± 0.3 μg $g^{-1}$. The survival of fry was similar in all groups, while the fry length and weight correlated positively with Se concentration in its body. In four-month-old fry the mean concentration of selenium in the fish body in the 16 Se group was 4.9 μg $g^{-1}$.

**Conclusion**
In Se-poor areas, immersion of fertilised eggs in water enriched with Se during egg swelling can be used to supplement the deficient element to fish. This innovative method can be implemented in aquaculture to improve breeding outcomes. Se concentration added to water should be adjusted to the selenium compound, fish species, and Se's content in the yolk.

In the sea trout studied, we recommend immersing fertilized eggs during swelling in Se concentrations of 4-8 mg $L^{-1}$ as $SeO_2$ for practical use in hatcheries.

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# OPTIMIZING OFFSHORE AQUACULTURE: USING REAL-TIME WAVE AND CURRENT DATA TO OPTIMIZE OPERATIONS AND MINIMIZE EXPENDITURES

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As aquaculture farms migrate further offshore, real-time environmental monitoring has become increasingly critical. This data informs farm design, feeding schedules, and ongoing operations in harsher conditions. Traditional monitoring systems are expensive, challenging to deploy and recover, and lack real-time capabilities for rapid decision-making.

The Spotter platform is a modular, rapidly deployable marine sensing buoy that delivers real-time surface and subsurface data to power better decisions. Users can attach up to two single-point acoustic Doppler current sensors at depths from 5 to 50 meters, ideal for net and mooring designs at aquaculture sites. The Spotter also supports temperature, pressure, dissolved oxygen, and hydrophone sensors, with a CTD and pH probe available by late 2025.

The Spotter Platform collects the wave and current data and transmits them in real time via satellite or cellular networks making them accessible through the Spotter Dashboard and API. The API seamlessly integrates with existing aquaculture specific software such as Aquacom, allowing entire organizations to leverage real-time data. Deploying multiple Spotter buoys allows for the co-location of current and wave measurements, enabling operators to make optimized decisions regarding site selection, allowing for a reduction in equipment failure risks, and improving feeding efficiency. Real-time access enables adaptive management, reducing downtime and increasing resilience to extreme weather. This equipment gives farm owners the ability to gain their NS9415 stamp.

The Spotter Platform provides a cost effective co-located monitoring solution that lowers capital expenditure for offshore aquaculture operations through real-time observations. Operating costs decrease with the reduction in cost for sensor deployment and installation, and improved decision-making for more streamlined maintenance, enhances productivity. Case studies will demonstrate cost benefits and operational improvements, reinforcing the value of real-time ocean data for optimizing offshore aquaculture.

# RACING TO THE FINISH: HOW EARLY-LIFE WARM REARING UNDERMINES CARDIORESPIRATORY PERFORMANCE AND HEAT TOLERANCE IN RAINBOW TROUT

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**Background**
In salmonid aquaculture, early-life rearing conditions can have long-term effects on fish performance and welfare. Elevated-temperature rearing regimes that promote rapid growth have been linked to increased post-stress mortality, possibly due to misshaped heart morphology observed in both fast-growing Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (Brijs et al., 2021; Frisk et al., 2020; Vindas et al., 2024). Additionally, farmed salmonids often exhibit coronary arteriosclerosis causing blockage of coronary artery flow and compromised myocardial oxygen and nutrient supply, a condition that is likely exacerbated by stressful rearing conditions (Brijs et al., 2021). The implications of these cardiovascular pathologies on cardiac and metabolic performance and stress resilience remain unclear. To address this, we investigated cardiorespiratory physiological and cardiac morphological consequences of warm rearing conditions in rainbow trout, raised under two temperature regimes at a commercial fish farm.

**Material and methods**
Warm- and cold-reared trout (n=32 per group) were raised under temperature regimes differing by 4–10°C for 7–8 months and then acclimated to a common temperature of 10°C for 4–7 weeks. Fish were surgically instrumented with electrodes to monitor heart rate via their electrocardiogram (ECG) and assess cardiac performance. After overnight recovery, heart rate and metabolic rate (via intermittent respirometry) were measured at rest and after an exhaustive chase protocol to attain maximum values. Aerobic scope and heart rate scope (maximum-resting values), reflecting the capacity to increase these performances above resting levels when needed (e.g., during stressful events), were calculated. The following day, fish were subjected to an acute warming protocol to determine critical thermal maximum ($CT_{max}$), after which they were euthanized for assessments of heart morphology. We evaluated the shape of the ventricle and the alignment of the *bulbus arteriosus*, and ongoing analyses will provide information about the prevalence and severity of coronary arteriosclerosis.

**Results**
Preliminary data show that warm-reared trout had significantly higher growth rates, more rounded ventricles (lower height:width ratio), and a misaligned *bulbus arteriosus* compared to cold-reared trout (Fig. 1A–C). Warm-reared fish also showed parallel elevations of resting and maximum heart rate (Fig. 1D). This led to a reduced heart rate scope. Furthermore, warm-reared trout had lower aerobic scope due to reduced maximum metabolic rate (Fig. 1E). Heat tolerance was also impaired in warm reared trout, as evidenced by a lower $CT_{max}$ (26.3°C vs. 27.3°C).

**Conclusions**
Our findings show that warm rearing accelerates early growth but induces cardiac remodeling linked to impaired heart function, reduced aerobic metabolic capacity, and lowered thermal tolerance in rainbow trout. These physiological impairments may contribute to elevated mortality in aquaculture, particularly during stress from *e.g.,* handling, treatment, and transport. Rearing protocols that prioritize growth should be re-evaluated, as they may compromise fish welfare and overall stress resilience. Ongoing experiments aim to further elucidate the long-term impacts of early-life thermal environments on adult trout health and performance.

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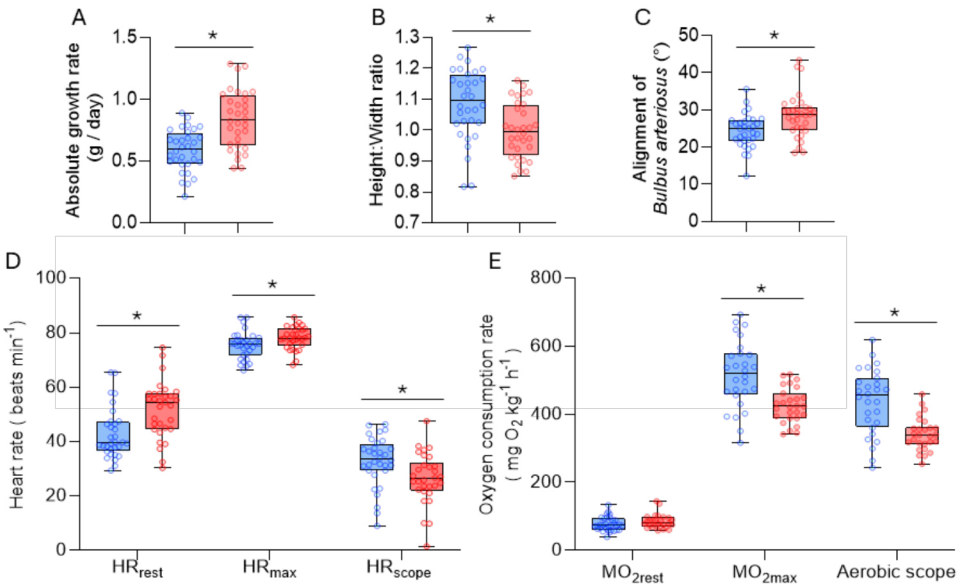
Figure 1. Effects of early-life rearing temperature (warm: red; cold: blue) in rainbow trout on A) growth rate, B) ventricular shape (height:width ratio), C) ventricle–*bulbus* alignment, D) heart rate (HR; resting, maximum, scope), and E) aerobic metabolic rate ($MO_2$; resting, maximum, scope).

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# FERMENTATION OF PLANT-BASED FEEDS WITH *Lactobacillus acidophilus* ACIDOPHILUS MODULATES MICROORGANISMS POPULATIONS IN WATER AND IN THE INTESTINE OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*) REARED IN BIOFLOC SYSTEM

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## Introduction

The use of fermented feeds for tilapia reared in a biofloc system (BFT) could have positive impact fish physiology and water microbiota. We hypothesize that plant-based fermented feeds will modulate the BFT microorganisms through interactions between the components of the fermented product and the heterotrophic microorganisms present in the water. The objective of this study is to acess the effect of a plant-based fermented feed on the count of microorganisms in the water and intestine of juvenile Nile tilapia (*Oreochromis niloticus*) reared in a biofloc technology (BFT) system.

## Materials and methods

Plant-based feeds for Nile tilapia, fermented for 6 and 18 hours, were compared with a positive control diet containing fishmeal and a negative control diet without animal protein. The experimental design was completely randomized, with four treatments and six replicates. The diets were isoproteic (33% CP) and isoenergetic (4100 kcal $kg^{-1}$). The plant-based feed was fermented with *Lactobacillus acidophilus* following a methodology adapted from Azarm and Lee (2014). Juvenile Nile tilapia (8.3 ± 0.2 g) were distributed into 24 tanks (70 L), with 17 fish per tank. After 60 days, water samples were collected from each tank for microorganism enumeration. Intestinal samples were also collected from three fish per tank for microorganism count analysis. Differences between results were assessed using analysis of variance (ANOVA) and Tukey's test ($P<0.05$).

## Results

Feed fermented for 18h increased ($P<0.05$) the acid lactic bacteria count in the water compared to fish meal and plant-based diets (Table 1). A reduction ($P<0.05$) in *Vibrio* sp. was observed in the water of the tanks that received the feed fermented for 6h relative to the fish meal diet. At the end of the trial, the concentration of heterotrophic bacteria in the intestines of Nile tilapia fed feed fermented for 6h was higher ($P<0,05$) than in the rest of the dietary groups. Levels of intestinal acid lactic bacteria were lower ($P<0.05$) in the fish that received plant-based feeds. The *Vibrio sp.* count was lower ($P<0.05$) Vibrionaceae family in the fish treated with fermented plant-based feeds.

Table 1. Microorganisms count in experimental tanks water and fish intestine after 60 days.

| | Fish meal | Plant-Based | Fermented 6h | Fermented 18h |
|---|---|---|---|---|
| *Lactic acid bacteria* | | | | |
| Water | 5.60±0.21b | 5.51±0.72b | 6.55±0.06ab | 6.65±0,10a |
| Intestine | 6.18±0.60a | 5.63±0.85b | 6.26±0.69a | 6.44±0.30a |
| *Heterotrophic microorganisms* | | | | |
| Water | 6.02±0,11 | 5.78±0,13 | 6.62±0,15 | 6.34±0,20 |
| Intestine | 5.25±0.89b | 5.82±0.36b | 6.37±0.33a | 6.00±0.66b |
| *Vibrio. sp.* | | | | |
| Water | 6.89±0,39b | 6.45±0,42ab | 4.89±1,38a | 5.49±0,45ab |
| Intestine | 6.80±0.55b | 6.60±075b | 5.12±0.70a | 5.30±1.04a |

Means folowed by diferent letters differs by Tukey test ($P<0,05$).

*(Continued on next page)*

**Discussion**

The use of fermented feeds positively affected the count of microorganisms in water in the BFT tanks. The increase in LAB during fermentation may have contributed to the reduction in *Vibrio sp.* The use of probiotics has already been demonstrated to be effective in BFT systems to control pathogenic bacteria in water (He et al., 2023). Regarding the bacteria counts in intestine, an increase in the count of heterotrophic bacteria and acid lactic bacteria was observed in fish fed with fermented feeds. The increase in intestinal acid lactic bacteria agrees with the increase count in water samples, since there is generally a sound correlation between the intestinal microbiota in fish and those of the environment (Turner et al., 2022). A similar modulation effect could have been observed in the reduction in *Vibrio sp*. through the feed and biofloc particles.

**Conclusions**

The use of plant based based fermented feeds increased the abundance of beneficial bacteria counts and reduced the pathogenic bacteria in the water of the BFT system and in the fish intestine.

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# FEED CO-FERMENTATION WITH *Lactobacillus acidophilus* AND *Lacticaseibacillus rhamnosus* IMPROVES FEED CONVERSION AND INTESTINAL HEALTH OF RAINBOW TROUT

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**Introduction**

Fermentation is a bioconversion or metabolic decomposition, carried out by microorganisms that transform complex substrates into simple compounds. During fermentation, secondary metabolites with prebiotic action are produced and the population of beneficial microorganisms with probiotic function increases, which can help control pathogens. Fermentation of feed can improve nutrient utilization (Yang et al., 2023) and intestinal health of fish (Neves et al., 2024). The objective of this work was to evaluate the effect of feed co-fermentation with *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus* on the growth performance and intestinal bacteria count of rainbow trout *Oncorhynchus mykiss* juveniles.

**Materials and methods**

Three times (3, 6 and 9h) of feed fermentation were evaluated in comparison with a control feed without fermentation. The experimental design was completely randomized with four treatments and four replicates. Juvenile rainbow trout (initial average weight of 22.2 ± 0.9 g) were distributed in 16 circular tanks with a useful volume of 500 liters at a density of 15 fish per tank. The tanks were interconnected to a recirculation system. A commercial feed for juvenile rainbow trout with 45% crude protein and 4 mm was fermented through solid-state fermentation (Azarn and Lee, 2014). At the end of the experiment (duration of 60 days) performance was evaluated and two animals per tank were collected for intestinal microorganism counts. Differences between results were assessed using analysis of variance (ANOVA) and Tukey's test ($P<0.05$).

**Results**

Fermentation of feed for 3 and 6 hours improved ($P<0.05$) the feed conversion of fish (Table 1). There was also a reduction in consumption ($P<0.05$), but growth performance was not affected ($P>0.05$). Fermentation of feed for 3 and 9 hours improved ($P<0.05$) lactic acid bacteria count in the intestine of fish (Table 2). Heterotrophic bacteria were not affected ($P>0.05$) by fermentation.

Table 1. Productive performance of rainbow trout fed fermented feed after 56 days.

| | Control | Fermented feed 3h | Fermented feed 6h | Fermented feed 9h |
|---|---|---|---|---|
| Final weight (g) | 60.52±6.07 | 65.07±3.85 | 62.16±3.42 | 58.54±3.92 |
| Daily feed ingestion (g) | 1.02±0.08a | 0.91±0.05b | 0.89±0.03b | 0.87±0.06b |
| Feed conversion | 1.56±0.06b | 1.28±0.09a | 1.33±0.10a | 1.49±0.07ab |
| Survival | 98.3±3.33 | 98.3±3.33 | 98.3±3.33 | 96.6±6.66 |

Table 2. Intestinal bacteria count of rainbow trout fed fermented feed after 56 days.

| | Control | Fermented feed 3h | Fermented feed 6h | Fermented feed 9h |
|---|---|---|---|---|
| Heterotrophic bacteria | 7.17±0.68 | 7.42±0.32 | 7.44±0.33 | 6.87±0.81 |
| Lactic acid bacteria | 5.03±0.13b | 5.31±0.08a | 5.18±0.10ab | 5.25±0.17a |

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**Discussion**

Fermentation improved feed efficiency of the feeds. The colonization of the digestive tract by probiotic bacteria associated with fermentation metabolites may have optimized the digestive process of fish that ingested fermented feed (Yang et al., 2023; Neves et al., 2024). Increased lactic acid bacteria count in the intestine confirms this result. Fermentation is an innovative and promising method that can contribute to the sustainability of aquaculture feeds.

**Conclusions**

Feed fermentation improved feed efficiency of rainbow trout juveniles. Greater colonization of lactic acid bacteria was also observed, indicating improved intestinal health.

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# EFFICIENT EXTRACTION OF POLYSACCHARIDES AND PROTEINS FROM *Saccharina latissima* USING ULTRASOUND-ASSISTED DEEP EUTECTIC SOLVENT METHOD: A SUSTAINABLE APPROACH FOR MARINE BIOMOLECULE ISOLATION

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## Introduction

Seaweeds are evolving as ample and sustainable sources of high-value compounds with prospective applications in food, feed, pharmaceuticals, and cosmetics. Brown seaweed *Saccharina latissima* is a robust source of proteins and polysaccharides. However, most existing extraction methods often comprise elevated energy input, extended extraction times, and use of hazardous solvents, which can degrade sensitive bioactive and other targeted compounds (Zhang et al., 2020). Deep eutectic solvents (DES), specifically those based on choline chloride and lactic acid, pose a green alternative, with exceptional biodegradability and solvating power (Dai et al., 2013). When combined with ultrasound-assisted extraction (UAE), which boosts mass transfer through cavitation, the process becomes vastly efficient for solute diffusion and cellular disruption (Chemat et al., 2017). This study explored the application of UAE-DES for the co-extraction of proteins and polysaccharides from brown seaweed biomass.

## Materials and Methods

Dried, ground *S. latissima* was pretreated with 80% ethanol to eliminate lipids, phenolics, and pigments, improving extraction accessibility. A DES was prepared using choline chloride and lactic acid (1:2 molar ratio), with 30% water added to lower viscosity and improve extraction efficiency. The mixture was heated at 60 °C to form a homogeneous solution. UAE was performed with a probe sonicator under variable process parameters of amplitude (70%, 80%, 90%), sonication time (5, 10, 15 min), and feed-to-solvent (F:S) ratio (3%, 5%, 7%), optimized with Box-Behnken design. Extracts were analysed for total protein content (TPC), protein recovery yield (PRY), total soluble carbohydrate (TSC), and carbohydrate recovery yield (CRY). Techniques such as FTIR, SEM, and HPLC were applied to characterize the extract's structural, thermal, and chemical features. Comparative studies were carried out using acidic water (pH 2), ultrasound alone and non-ultrasound methods to determine the competence of DES and UAE.

## Results and Discussion

### Effect of Ultrasound Amplitude

A clear enhancement in extraction efficiency was observed with increasing amplitude. At 90% amplitude and 5 min sonication, TPC and PRY were significantly higher (729.75 µg/mL and 13.32 mg/g, respectively) compared to 70% amplitude (585.75 µg/mL TPC, 9.97 mg/g PRY). The enhancement is credited to effective cavitation and cell disruption, accelerating solubilization of intracellular compounds (Chemat et al., 2017).

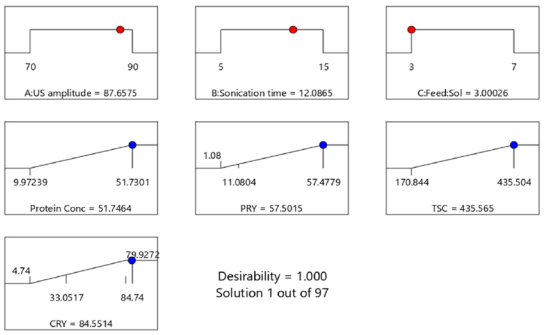


Figure 1: Optimized conditions predicted by Response Surface Methodology (RSM) modeling for the target process parameters.

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**Influence of Sonication Time**

Prolonged sonication times increased yield, remarkably at increased amplitudes. At 90% amplitude and 15 min treatment, PRY rose to 15.08 mg/g and CRY to 53.33%. However, overexposure without an optimal F:S ratio could lead to compound degradation, highlighting the necessity for a balanced treatment regime.

**Feed-to-Solvent Ratio Effects**

At 3% F:S ratio, yields were increased under optimized amplitude and time, with CRY extending 84.25%. Although a lower solvent volume guarantees more intense solute-solvent interaction, optimal extraction was mostly achieved at 5% ratio, where adequate diffusion and solubilization occurred without dilution effects.

**Optimization and Predicted Responses**

Using response surface methodology (Box-Behnken), the optimal conditions were: 88% amplitude, 12 min sonication, and 3% F:S ratio, yielding predicted responses of TPC: 51.74 mg/g, PRY: 57.50%, TSC: 435.56 mg/g, and CRY: 84.55%. Experimental validation confirmed these trends.

**Comparative Efficiency of Extraction Methods**

Compared to acidic water extraction and ultrasound extraction alone, UAE-DES demonstrated significantly higher yield and purity. Non-ultrasound DES methods, though more efficient than conventional solvents, were subsidiary to UAE-DES, confirming the interactive benefit of cavitation-enhanced solubilization.

**Conclusion**

The research confirms UAE-DES as an advanced, efficient, and green method for the co-extraction of polysaccharides and proteins from brown seaweed. The optimized factors led to significantly greater yields and improved purity of extracts associated with the conventional method. The DES system, being biodegradable and tunable, poses a sustainable extraction platform aligned with green chemistry principles. These protein- and polysaccharide-rich extracts display elevated potential for application in aquatic and terrestrial animal feed formulations, as well as in functional foods and nutraceuticals, supporting the valorisation of marine biomass within circular bioeconomy frameworks.

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# MITIGATING CARP FARMING IMPACTS ON BIODIVERSITY

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## Introduction

Freshwater habitats, such as rivers, lakes, and wetlands, host approximately one-third of all vertebrate species while covering less than 1% of the Earth's surface. With such high biodiversity, freshwater ecosystems, compared to terrestrial and marine environments, are more vulnerable and endangered to biodiversity losses, declining ecological function, decreasing natural benefits, and climate change. The relationship between freshwater aquaculture and biodiversity is complex, presenting opportunities and challenges. When managed sustainably, freshwater aquaculture can contribute to reduced pressure on wild fish stocks, habitat restoration (regenerative aquaculture), conservation of endangered species, and support ecosystem services, thus enhancing global and local biodiversity.

During the common carp (*Cyprinus carpio*) production season, earthen ponds accumulate more nutrients than are discharged to the river system. However, seasonal draining of the ponds requires the transfer or harvest of fish (Oct–Dec), which mobilises marked amounts of sediment settled in the bottom of the pond. This often leads to adverse effects on the water quality and biodiversity of the downstream environment. The study aimed to evaluate the impact of common carp farming on the biodiversity of macroinvertebrates and benthic diatoms. The study also aimed to assess the survival of bioindicator species installed downstream of the farm while implementing SAFE filtering technology.

## Materials and Methods

The study was conducted at the ICR carp farm in NW Poland, located on the Piaskowa river, one of the tributaries of the Rega river that opens to the Baltic Sea at Mrzeżyno. The biodiversity assessment involved a comparative evaluation of the species diversity of benthic macroinvertebrates across different seasons (winter, spring, and summer of 2023 and 2024). The overall impact of the common carp farm on diatom communities was assessed above and below the farm. Finally, three bioindicator species (noble crayfish, *Astacus astacus*; thick-shelled mussel, *Unio crassus*; and river water-crowfoot, *Ranunculus fluitans*) were installed below the discharge channel at the time of pond drainage and sediment sequestration with the SAFE filtering system.

Benthic samples were collected using kick sampling from a 1 m$^2$ cross-section area of the river at sampling points. Next, samples were sieved through a 0.5 mm mesh, stored, and delivered to the laboratory, where benthic organisms were separated from the sediment, identified, and preserved in 70% alcohol.

Diatom samples were taken with a toothbrush from the upper surfaces of gobble-sized stones collected from the stream bottom. The sample material from the stones (n = 10) was pooled into one sample. The samples were stored in alcohol and then sent to the laboratory of the Finnish Environment Institute for further processing. In the laboratory, the samples were cleaned of organic material with a strong acid solution. Permanent slides were made to count and identify the diatom individuals.

The primary biodiversity metric employed in the comparative analysis was the Shannon-Wiener diversity index (H'). The method developed in Zar (2010) was applied to assess the significance of differences. The analysis was conducted using the sample-size-based rarefaction and extrapolation (R/E) sampling curve, which presents diversity estimates with confidence intervals as a function of sample size.

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**Results and Discussion**

The biodiversity level downstream on the farm was significantly higher after implementing the SAFE filtering technology, which significantly reduced the emission of sediments from the ICR carp farm. Comparative analysis using sample-size-based R/E sampling curves showed that after the discharge process ceased in early winter 2024 (SAFE filtering), interpolated species diversity indicated a significant increase ($p = 0.007$) in benthic organisms' diversity downstream of the farm in 2024, when compared to 2023. Extrapolated predictions further strengthen this observation. Similar significant trends were observed for comparisons obtained for spring ($p = 0.041$) and summer ($p < 0.000$) seasons. These results indicate that minimising sediment release by implementing SAFE filtering technology can contribute to better regeneration of the river ecosystem in early spring, thus helping maintain biodiversity in the freshwater environment.

Taxon richness and H' diversity indices for benthic diatoms were lower in the inflow channel above the farm, compared to the discharge channel of the ICR carp farm. Surprisingly, the proportion of abnormal forms was higher in the inflow than in the outflow channel. This indicates that the carp farm does not increase the proportion of abnormal diatom forms during the production season. The 10 most common diatom taxa varied between the inflow and outflow channels. In winter, *A. minutissimum s.l.* was the most common taxon in the inflow channel, closely followed by *P. lanceolatum* (prefers eutrophic, alkaline conditions)*, A. minutissimum*, and *P. frequentissimum* – together they formed 79% of the diatoms present in the inflow channel. In the same period, the five most common taxa in the discharge channel were planktic taxa from the *Stephanodiscus*, *Cyclostephanos* and *Aulacoseira* genera. Overall, the inflow channel was generally more dominated by one or two taxa, contrary to the discharge channel.

Even with SAFE filtering technology, the discharge of post-production waters into the Piaskowa River caused adverse effects. For instance, some experimental cages were damaged or buried in sediments. The tearing and perforation of the cages by objects carried by the river influenced the distortion of the noted bioindicators' survival rate. In particular, noble crayfish might have escaped from damaged cages. However, in one of the undamaged cages, a 100% survival rate of noble crayfish was observed, indicating that such a sensitive organism could survive when SAFE filtering technology was implemented. Thick-shelled mussels have limited mobility on the riverbed, making it unlikely that they could escape from damaged experimental cages. The main cause that reduced the survival rates of these animals was the organic and inorganic material that filled the cages. The mortality of thick-shelled mussels downstream of the discharge channel was significantly higher (33.4%) than the control group of mussels located upstream (0%; $p = 0.037$). The selection of the plant bioindicator, river water-crowfoot, proved to be too sensitive to habitat conditions in the Piaskowa River, resulting in 100% mortality regardless of nutrient discharge. SAFE filtering technology allowed the survival of two bioindicator species that had not been observed before in the Piaskowa River, in the most challenging period of the year, i.e., water discharge during fish harvesting.

These findings highlight the potential of SAFE filtering technology to mitigate the negative impacts of sediment discharge during aquaculture operations, supporting macroinvertebrate and diatom biodiversity. While challenges remain in fully safeguarding sensitive species, the technology represents a promising step toward more biodiversity-friendly freshwater aquaculture practices.

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# IMPACT OF DIETARY INCLUSION OF BLACK SOLDIER FLY LARVAE MEAL ON THE GUT MICROBIOME OF ATLANTIC SALMON: INSIGHTS FROM FIRST FEEDING AND SMOLTIFICATION TRIALS

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Black Soldier Fly larvae meal (*Hermetia illucens*; BSFLM) is emerging as a sustainable and functional alternative to fishmeal in aquaculture diets. This study investigated the effects of graded dietary inclusion levels of defatted BSFLM on the gut microbiome of Atlantic salmon (*Salmo salar*) at two critical ontogenetic stages: first feeding and smoltification. Fry were fed diets containing 0%, 8%, 16%, or 24% BSFLM for 21 days during the first feeding stage, while pre-smolts received diets with 0%, 5%, or 15% BSFLM during smoltification. Microbial communities from the gut, feed and water were characterised using 16S rRNA gene amplicon sequencing, incorporating a novel titration-based quantification step to enhance library preparation accuracy.

Results highlighted that BSFLM inclusion during the first feeding significantly modulated gut microbiome composition, with distinct community structures observed across diets (Figure 1; PERMANOVA, $p$ = 0.001). BSFLM diets were associated with increased microbial richness and evenness, suggesting enhanced diversity during early gut colonisation. Taxonomic profiling further revealed diet-specific shifts in the relative abundance of key bacterial taxa.

During smoltification, BSFLM inclusion also altered the gut microbiome, with all insect meal diets exhibiting consistently higher microbial richness compared to the control. Taxonomic analyses revealed diet- and time-dependent shifts in microbial composition, reflecting dynamic restructuring of the gut microbiome throughout smoltification.

These findings demonstrate that BSFLM not only serves as a sustainable alternative to fishmeal but also exerts stage-specific modulatory effects on the gut microbiome of Atlantic salmon, with potential implications for fish health and performance.

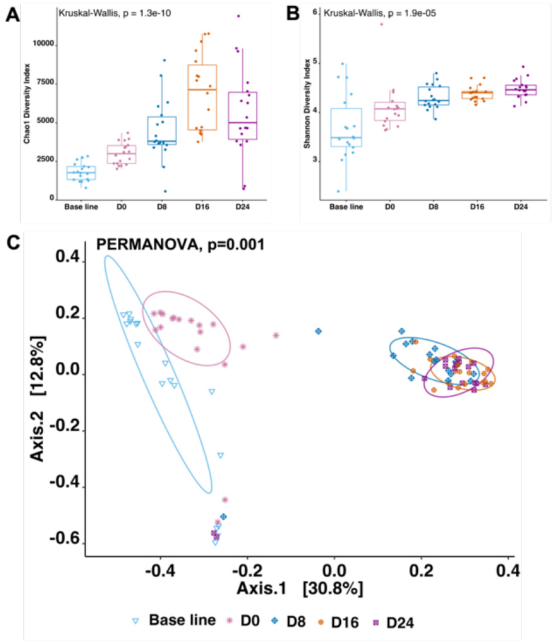


Figure 1. Gut microbiome diversity and composition of Atlantic salmon fry fed diets containing graded levels of BSFLM during the first feeding trial. (A) Alpha diversity metrics showing observed species richness (Chao1 index) and (B) evenness (Shannon index) across different diets (0%, 8%, 16%, and 24% BSFLM inclusion). Significant differences were detected by Kruskal-Wallis tests ($p$ < 0.001). (C) Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity of gut microbial communities, illustrating distinct clustering by diet (PERMANOVA, $p$ = 0.001), indicating that BSFLM inclusion significantly influenced overall community structure.

# ADVANCING TILAPIA WELFARE IN EGYPTIAN AQUACULTURE THROUGH A ONE WELFARE LENS

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Improving fish welfare in low- and middle-income countries is often framed as a trade-off with productivity. Yet in Egypt, the world's sixth largest producer of farmed *tilapia*, we find evidence that better welfare can go hand-in-hand with improved performance and farmer resilience. This work presents a One Welfare case study of efforts to improve *tilapia* welfare in semi-intensive pond systems in the Nile Delta, where farms are increasingly transitioning to more intensive practices in response to climate and economic pressures.

We describe the rollout of the FAI Tilapia Welfare App—a farmer-friendly digital tool for scoring fish welfare indicators on site—and the lessons learned from its uptake and use across small and medium farms. Drawing on survey, observational and farmer feedback data collected by Ethical Seafood Research and partners from 2022 to 2025, we explore the links between welfare scores, farm conditions, and farmer perceptions, and assess the tool's practical impact on management. The results suggest that improvements in welfare, such as reduced handling stress and more humane fasting and harvest practices, are feasible with minimal investment and can be aligned with existing biosecurity and quality goals.

We argue that centring welfare within a One Welfare framework—considering fish, farmer and environmental wellbeing together—helps build a stronger case for change. This approach is relevant not just to Egypt but to other African aquaculture contexts facing similar intensification trajectories.

# OPTIMISING LARVAL NUTRITION IN FLATFISH USING PROTEIN HYDROLYSATES: GROWTH AND REDOX EFFECTS

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## Introduction

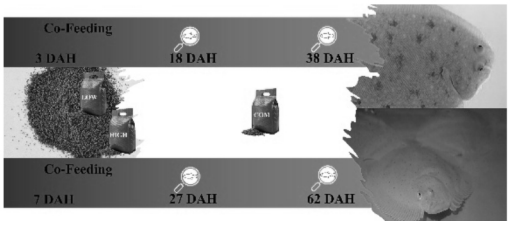
The farming of Senegalese sole (*Solea senegalensis*) and turbot (*Scophthalmus maximus*) is expanding driven by market demand and supported by advances in hatchery technology, including novel microdiets. However, optimising early-stage nutrition remains a major challenge. Live feeds, commonly used at first feeding, are nutritionally suboptimal—particularly regarding protein quality—and have been associated with inconsistent larval growth. Formulated microdiets, used to supplement live feeds in co-feeding regimes or to support earlier weaning, have been proposed as a solution, provided they are adapted to the limited digestive capacity of these altricial larvae. At mouth opening, fish larvae immature digestive tract lacks a functional stomach and gastric glands, limiting their ability to digest complex proteins such as fishmeal. Protein hydrolysates (PH), rich in free amino acids and small peptides, offer a more digestible protein source and have been shown to improve growth and survival in various marine fish species. The inclusion of PH from first feeding may support larvae through metamorphosis and improve long-term robustness, however their peptide profile needs to be optimized, to facilitate digestion, while preventing excessive leaching of amino acids into the water, and also an overload of the absorption and metabolic processes.

## Material and Methods

Two trials were conducted at CCMAR facilities under optimal abiotic conditions using recirculating aquaculture systems. Two dietary treatments were tested in triplicate: LOW (a commercial-like diet rich in a low molecular weight PH) and HIGH (a similar diet rich in high molecular weight PH). Larvae were co-fed from mouth opening - 3 days after hatching (DAH) for Senegalese sole and 7 DAH for turbot - following CCMAR's Aquaculture Research Group protocol. At the end of the pelagic phase (18 DAH for sole and 27 DAH for turbot), larvae were sampled to assess growth, survival, and redox status. Fish were then transferred to benthic rearing systems and fed a commercial-like diet until final sampling at 38 DAH (sole) and 62 DAH (turbot).

## Results

During the pelagic phase, Senegalese sole larvae fed the HIGH diet showed significantly greater growth, suggesting a positive correlation between hydrolysate molecular weight and performance. In contrast, turbot larvae grew better on the LOW diet, indicating an inverse relationship. Redox status was unaffected at the end of the pelagic phase in both species. After metamorphosis, growth remained similar across treatments. However, in turbot, fish previously fed the LOW diet displayed higher total glutathione (GH) and catalase (CAT) activity, along with reduced lipid peroxidation (LPO), suggesting enhanced antioxidant capacity and improved cellular protection.

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**Conclusions**

The inclusion of protein hydrolysates from mouth opening influenced early growth in a species-specific manner, with opposing effects observed in Senegalese sole and turbot. While redox status was unaffected during the pelagic phase, differences in antioxidant markers post-metamorphosis in turbot suggest that early dietary interventions may shape long-term physiological robustness. These findings highlight the potential of tailored hydrolysate-based diets to optimise early nutrition and resilience in flatfish aquaculture.

**Acknowledgments**

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# EFFECTS OF KRILL MEAL ON PHENOMIC RESPONSES AND BLOOD PROTEOMICS IN RAINBOW TROUT REARED IN A CHALLENGING HIGH-TEMPERATURE

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## Introduction

Temperature is a critical factor in climate change, with its effects becoming particularly pronounced during heatwaves, defined as periods when the mean or maximum temperature increases by at least 3–5°C for a maximum of three consecutive days. Such conditions significantly impact aquatic environments, often resulting in mass mortalities. The reoccurrence of heatwaves poses a substantial challenge for the salmon and rainbow trout industry. There exists a strong correlation between the heat resistance of fish and their diet. Krill meal (KM) offers a well-balanced amino acid profile akin to fish meal, along with high levels of phospholipids, n-3 polyunsaturated fatty acids (n-3 PUFAs), astaxanthin, vitamins, and minerals. Consequently, KM has the potential to alleviate heat stress in cultured fish due to its functional nutrient content. This study aims to evaluate the phenomic responses rainbow trout fed experimental diets containing 0%, 5%, 7.5%, and 10% krill meal (KM) under a challenging high temperature (14°C-control, 14°C→21°C, and 21°C→23°C). The blood proteome of rainbow trout fed control, KM7.5 and KM10 diets was also assessed to understand the role of the altered proteins in the fish's metabolic and immune systems when maintained at 21°C→23°C.

## Material and Methods

Female rainbow trout juveniles (61.8 g) were placed into two RAS with 16 cylindrical tanks (4 replicates per dietary group) of 350 L capacity at 25 fish/tank. Five isonitrogenous (46% CP) and isolipidic (28% CL) diets were formulated using KM (0, 5, 7.5, and 10 mg/kg; purity: 99%, Aker Qrill Company). Fish were fed twice daily until apparent satiety. During Phase I, four experimental diets were randomly assigned to four replicate tanks using a randomized block design across two RAS. Throughout the 70-day period, fish were manually fed to apparent satiation at 14°C. Following Phase I, one RAS system was designated as the heat stress group. The water temperature of two tanks per experimental group was increased by 1°C daily until reaching 21°C, while the remaining tanks in the second RAS were maintained at 14°C as a control. During Phase II (14°C→21°C), fish continued receiving the same diets, and feed intake was recorded for 7 days. Subsequently, the water temperature in the heat stress group was increased from 21°C to 23°C over 3 days, and the fish were kept at this temperature for 7 more days (Phase III, 21°C→23°C). At the conclusion of each phase, three fish were sampled for fillet fatty acid and proximate composition analysis. Blood samples were only collected for proteomic analysis after Phase III. All data were statistically compared with a two-way ANOVA, where a 4×2 factorial design was followed with diet and heat stress temperature as fixed factors.

## Results

At the conclusion of Phase I (14°C), the final weight (g/fish) and thermal growth co-efficiency (% TGC) in the KM5 group were numerically higher than the control, while there was no significant difference between the KM5 and KM10 fed fish. Overall, no significant differences in feed intake or feed conversion ratios (FCR) were observed across the different diets during Phase I. In Phase II (14°C→21°C), there was a discernible trend showing increased growth in fish fed with KM5 at 21°C compared to those maintained at the control temperature of 14°C. Regardless of the dietary treatment, TGC was significantly higher in fish fed the experimental diets at 21°C than in the fish that had remained at 14°C. In Phase III (21°C→23°C), the final growth of fish fed at 14°C was greater than that of fish fed at 23°C irrespective of the dietary treatments. Fish fed with KM5 and KM7.5 showed higher growth than those fed KM10 during Phase III (21°C→23°C). While fatty acid composition remained unchanged in Phase I, EPA increased in KM5, KM7.5, and KM10 at 21°C. Docosahexaenoic acid (DHA) was sensitive to heat and increased in fish kept at 21°C, irrespective of dietary treatment. A similar trend was observed in fish maintained at 23°C in Phase III. The DHA/EPA ratio was significantly affected by diet and heat stress; however, no significant interaction

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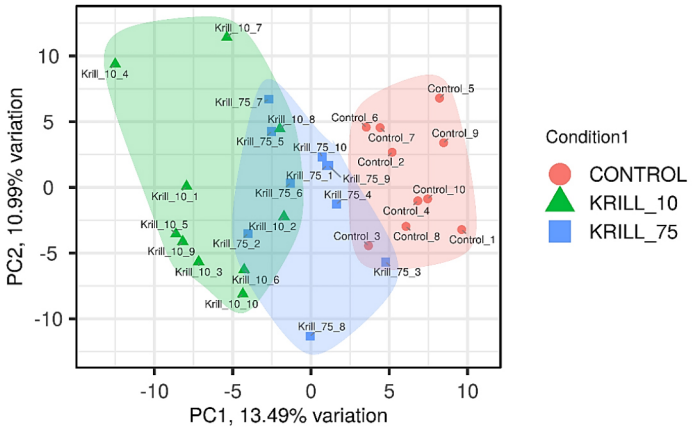


**Fig 1.** Principal Component plot of the blood proteome samples from the three feeding groups of rainbow trout.

effects were observed. Proteomic analysis of rainbow trout exposed to KM7.5 and KM10 revealed significant physiological adaptations (Fig. 1). The higher doses of krill meal (KM7.5 and KM10) elicited stronger upregulation of oxygen transport proteins, particularly haemoglobin subunit alpha-1, and showed comprehensive activation of complement system components, with six proteins significantly upregulated, indicating a better coping ability of trout under challenging temperature, which correlated well with improved survival in KM diets, displaying a dose response, with KM10 resulted in the highest survival at 23°C. Functional analysis of the differentially expressed proteins in the blood proteome will be presented.

**Discussion**

The observations of the present study support the clear benefits of KM in the rainbow trout diets under heat-stress conditions with better growth and survival. The primary phenomic responses to KM supplementation are intake-linked and their benefits when used in diets under heat stress. Based on blood proteomic analysis, these results demonstrated a robust cellular response to both the KM7.5 and the KM10 interventions that was not reflected in the growth performance of the fish. This body of work notably makes a strong case for further exploring the use of KM in aquafeeds as a functional ingredient to improve robustness in rainbow trout under heat stress. It is essential to further investigate this aspect to achieve a more comprehensive understanding of the potential benefits of KM inclusion, particularly concerning the purported enhancement of oxygen-carrying capacity under heat stress in actual culture conditions.

# SEASONAL VARIATION IN THE EXPRESSION OF SPECIFIC HEALTH-RELATED GENES IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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## Introduction

Understanding the physiology of fish raised in cages is essential to assess their current condition in response to shifting environmental factors, develop protective measures, and enhance their resilience during these times. By boosting the fish's defense mechanisms and minimizing oxidative stress, these adverse effects can be mitigated. In this regard, seasonal sampling was conducted in the cages of a commercial operation chosen as a representative from the Milas region, the most densely populated aquaculture area in Türkiye. Through molecular-level analyses, the health status of the European sea bass (*Dicentrarchus labrax*) was comprehensively assessed.

## Material and Methods

To examine health-related gene expression in European sea bass across seasons, samples were collected quarterly from cages in August, November, February, and June during 2023–2024. Fish were sourced from Gümüşdoğa company's cages in Kıyıkışlacık (Milas, Muğla, Türkiye). Fish of similar weights (~80-120 g) were selected to reduce sampling variability. Twenty-four fish were randomly sampled under anesthesia, with 12 from two cages per season (n=12, N=24 fish/season). Liver tissues from 12 fish, 6 from each cage, were analyzed per season (n=6, N=12 fish/season). Total RNA was extracted from liver using TRIzol reagent (Thermo Fisher Scientific) following manufacturer's guidelines. GAPDH served as the reference gene. Expression levels were assessed using the $2^{-\Delta\Delta Ct}$ method, based on threshold cycle (Ct) values. Gene expression differences were analyzed using Duncan's test and Kruskal Wallis with Mann–Whitney U tests for homogeneous and inhomogeneous data. Data are reported as mean ± standard error, with significance at $P<0.05$.

**Table 1.** Seasonal variation in some health-related gene expressions in liver tissues in European sea bass. Values are represented as mean and standard error. Data with different letters are significantly different among seasons ($P < 0.05$).

| | Seasons | | | |
|---|---|---|---|---|
| **Genes** | **Spring** | **Summer** | **Autumn** | **Winter** |
| Hepcidin | $2.44 \pm 0.78^{A}$ | $0.85 \pm 0.54^{B}$ | $0.45 \pm 0.14^{B}$ | $1.45 \pm 0.26^{A}$ |
| HSP70 | $1.65 \pm 0.47^{A}$ | $0.20 \pm 0.12^{B}$ | $0.15 \pm 0.06^{B}$ | $0.51 \pm 0.08^{A}$ |
| HSP90 | $1.76 \pm 0.52^{A}$ | $0.48 \pm 0.32^{B}$ | $0.25 \pm 0.07^{B}$ | $0.77 \pm 0.20^{A}$ |
| SOD | 2.02 ± 0.84 | 1.27 ± 0.74 | 0.86 ± 0.27 | 2.01 ±0 .38 |
| CAT | 1.13 ± 0.16 | 1.02 ± 0.15 | 0.55 ± 0.05 | 0.90 ± 0.25 |
| GPX | $2.30 \pm 0.69^{A}$ | $0.51 \pm 0.32^{B}$ | $0.32 \pm 0.11^{B}$ | $1.07 \pm 0.20^{A}$ |
| DCNT | 1.25 ± 0.39 | 0.34 ± 0.11 | 0.25 ± 0.07 | 0.66 ± 0.24 |
| CAS8 | $1.85 \pm 0.53^{A}$ | $0.12 \pm 0.03^{B}$ | $0.19 \pm 0.06^{B}$ | $0.76 \pm 0.17^{A}$ |
| CAS9 | $2.19 \pm 0.65^{A}$ | $0.27 \pm 0.08^{B}$ | $0.47 \pm 0.16^{B}$ | $1.55 \pm 0.27^{A}$ |
| IGF1 | $1.79 \pm 0.56^{A}$ | $0.14 \pm 0.09^{B}$ | $0.07 \pm 0.02^{B}$ | $0.47 \pm 0.13^{A}$ |
| IGF2 | $2.44 \pm 0.74^{A}$ | $0.30 \pm 0.21^{B}$ | $0.17 \pm 0.05^{B}$ | $0.81 \pm 0.29^{A}$ |
| GH | $1.40 \pm 0.44^{A}$ | $0.27 \pm 0.18^{B}$ | $0.14 \pm 0.05^{B}$ | $0.64 \pm 0.17^{A}$ |

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**Results**

Our findings indicate that the expression levels of IGF1, IGF2, and GH genes were notably elevated during the spring and winter months compared to the summer and autumn (P < 0.05). Similarly, hepcidin gene expression was significantly higher in spring and winter than in summer and autumn ($P<0.05$). In contrast, no seasonal variation was detected in the expression of the DCNT gene ($P>0.05$). Additionally, there was no significant seasonal difference in the expression of SOD and CAT genes ($P>0.05$). However, GPX gene expression was more pronounced in spring and winter than in summer and autumn ($P<0.05$). It was also noted that the expression levels of HSP70 and HSP90 were significantly greater in spring and winter compared to summer and autumn ($P<0.05$). Likewise, CAS8 and CAS9 gene expressions were significantly higher in spring and winter than in the summer and autumn months ($P<0.05$). All data are presented in Table 1.

**Discussion**

The study's results revealed that the genes associated with growth, such as GH, IGF1, and IGF2; the antimicrobial peptide hepcidin; the antioxidant enzyme GPX; stress-related genes HSP70 and HSP90; and apoptosis-related genes CAS8 and CAS9, were highly expressed during spring and winter. The data clearly indicate that fish health deteriorates in these seasons. This is believed to be linked to alterations in water quality parameters, particularly temperature. For instance, it is known that the thermocline layer in the Aegean Sea shifts in spring, leading to fish experiencing sudden temperature changes along the cage column. This scenario suggests that temperature fluctuations directly or indirectly induce stress in fish, making them more susceptible to diseases. Conducting more detailed research based on this information will enhance aquaculture efficiency and aid in developing various strategies for these specific seasons.

**Acknowledgments**

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# EFFECTS OF DIETARY VITAMIN C AND COBALT CHLORIDE ON HEMATOLOGICAL PARAMETERS IN *Acipenser baerii*

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**Introduction**
Vitamin C and cobalt are essential micronutrients for aquatic animal health. Vitamin C boosts immunity and growth, requiring dietary intake in most fish (Gouda *et al*., 2020). Hematological assessments are vital for fish health monitoring. Cobalt, as a coenzyme, supports vit $B_{12}$ synthesis, crucial for metabolism and preventing anemia. Limited studies exist on combined effects of vit C and cobalt in sturgeon (Li *et al*., 2022). This study examines their combined effects on hematological indices in Siberian sturgeon.

**Materials and Methods**
270 juvenile Siberian sturgeon were reared for 12 weeks in 27 tanks, using a factorial design with $CoCl_2$ (2, 4 mg/kg) and vit C (200, 800 mg/kg). Blood samples were collected and analyzed for RBC, WBC, differential leukocytes, Hb (cyanmethemoglobin method), HCT. Data were analyzed by two way-ANOVA and Duncan's test (SPSS).

**Results**
The results of the blood indices showed that the inclusion of 800 mg of vit C per kg of diet significantly increased the white blood cell count compared to the control and other treatment groups. The control group exhibited the lowest white blood cell count ($p < 0.05$). Additionally, the levels of RBC, Hb, and Hct in fish fed a diet that included a combination of 2 mg of $CoCl_2$ and 200 mg of vit C were significantly higher than those in the other treatments and the control group.

**Discussion**
In the present study, the addition of $CoCl_2$ and vit C to the diet of Siberian sturgeon significantly improved hematological indices. Given that hematological indices are crucial measures for assessing fish physiological health, this study observed significant changes in the number of WBCs, RBCs, Hb, Hct, and differential leukocytes. Specifically, the number of WBCs and RBCs increased in the treatments containing these substances, with the highest levels of WBCs observed in the 800 mg/kg vit C treatment and the highest levels of RBCs in the 2 mg/kg $CoCl_2$ + 200 mg/kg vit C treatment. This increase indicates a positive effect of these substances on hematopoiesis and the strengthening of the fish's immune system. These effects are attributed to the role of cobalt chloride in the synthesis of vit $B_{12}$ (Tonye & Sikoki, 2014) and the role of vit C in iron absorption and erythropoiesis (Garcia et al., 2007). Vitamin $B_{12}$ aids in hematopoiesis by increasing the production and maturation of RBCs, while vit C facilitates iron release from the liver and improves its absorption (Hall & Hall, 2020). These results are consistent with previous studies on the effects of these substances on hematological indices in other fish species (Makwinja & Geremew, 2020). Furthermore, the 2 mg/kg $CoCl_2$ + 200 mg/kg vit C treatment increased Hb and Hct, indicating improved oxygen-carrying capacity. Micronutrient deficiencies can lead to anemia (Brunt & Austin, 2005). The increase in Hb/Hct is associated with ferritin release by vit C, although Hb changes may also stem from osmotic stress. Vitamin C enhances iron absorption. Differences from other studies (Narra et al., 2015) may be species-specific. These findings highlight the complex role of vit C and $CoCl_2$ in the hematology and health of Siberian sturgeon.

Hematological indices in *Acipenser baerii*, fed diets containing cobalt chloride and vitamin C after 12 weeks.
Significant difference $p < 0.05$ are indicated by non-synonymous letters, and asterisks (*) denote significant interaction effects $p < 0.05$.

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# BENTHIC DIVERSITY CHARACTERIZATION OF THE PIEDRAS RIVER: BASIS FOR THE RECOVERY OF THE CARPET SHELL (*Ruditapes decussatus* Linnaeus, 1758)

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Introduction

The MOLUSOS project aims to revitalise bivalve aquaculture and restore natural populations of the carpet shell clam (*R. decussatus*) in the estuary of the Piedras River (Huelva, SW Spain), where this species has experienced a pronounced decline in recent decades, possibly associated with overfishing, the expansion of the recreational boating fleet, and habitat degradation. To achieve this goal, three strategies have been proposed: (1) study of the current benthic fauna and sediment conditions in the Piedras River; (2) establishment of a broodstock sanctuary within the estuary; and (3) monitoring of *R. decussatus* populations to assess the effects of the sanctuary. The project is currently in its first phase.

This study is being conducted to characterise benthic diversity in the Piedras River estuary and to establish a baseline for restoration actions targeting *R. decussatus.*

Materials and Methods

In this initial phase, a baseline survey of the benthic fauna of the Piedras River was conducted, comprising 38 sampling stations distributed along a 12 km stretch of the river. Sampling was performed using a boat-towed dredge. The study area encompassed four estuarine sectors that have historically supported *R. decussatus* populations: San Miguel (SM), with 12 stations distributed between the main channel (R) and the shore (V); Casa del Palo (CP), with 14 stations across the channel and shore; La Chanca (CH), with 7 stations located in the channel; and Tartesos (T), with 5 channel stations. At each station, three replicates were collected and the species and/or morphotypes present were identified taxonomically. Descriptive analyses were performed in Microsoft Excel, while diversity indices (Shannon–Wiener, Simpson, and species richness), similarity analyses—including NMDS (Non-metric Multidimensional Scaling) based on the Bray–Curtis index and Multilevel Pattern Analysis were conducted in R software.

Results

Data from the SM, CP and T sectors were processed and analysed, yielding species and morphotype abundances (ind/m$^2$), diversity indices for each sector (Table 1), and relative composition (%) by taxonomic group, as well as the proportion of molluscs and commercial bivalves identified (Table 2).

The similarity analysis (Figure 1) revealed three main groupings among sampling stations, identified through NMDS and assessed using the Silhouette index. In addition, the indicator index for each species was estimated for each group, which made it possible to determine their characteristic species: Group 1 dominated mainly by *C. glaucum* and *R. decussatus* as highly indicative species (IndVal > 0.95, p < 0.005); Group 2 represented exclusively by *S. solida* and *V. verrucos*a as indicator species (IndVal = 0.723, p <0.05); and Group 3 represented mainly by *Parvicardium exiguum*, *Tritia reticulata* and *Abra alba* (IndVal >0.95, p<0.005).

**Discussion**

The results indicate that SM-V exhibited the highest diversity and species richness, together with a more even distribution of abundances. CP showed intermediate values, with a slight reduction in CP-R compared to CP-V. In contrast, T recorded the lowest diversity and richness, with a marked dominance of a few species. These differences highlight a significant variation in benthic community structure among sectors, likely associated with local environmental factors (e.g., substrate quality, hydrodynamics, anthropogenic pressure). Overall, the findings suggest that SM provides more favourable conditions for sustaining diverse and even benthic communities, whereas T offers an environment that supports more simplified communities dominated by a few species.

*(Continued on next page)*

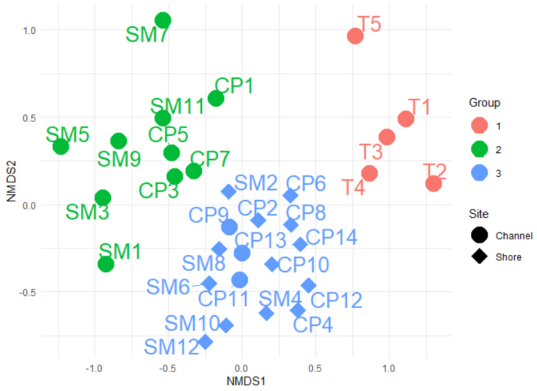
Table 1. Diversity indices for each study sector.

| Sectors | Shannon | Simpson | Riqueza | Equitatividad |
|---|---|---|---|---|
| SM-V | 3.215 | 0.924 | 63 | 0.776 |
| CP-V | 2.659 | 0.876 | 50 | 0.68 |
| SM-R | 2.573 | 0.882 | 32 | 0.742 |
| CP-R | 2.546 | 0.881 | 47 | 0.661 |
| T | 1.034 | 0.515 | 15 | 0.382 |

Table 2. Relative abundance (%) of taxonomic groups (>3%) by sector

| TAXONOMIC GROUP | | SECTORS | | |
|---|---|---|---|---|
| | | SM | CP | T |
| **Taxa** | Annelida | 9.84 | 15.44 | 5.93 |
| | Arthropoda | 26.44 | 16.05 | 8.96 |
| | Ascidiacea | - | 15.44 | - |
| | Echinodermata | 2.67 | - | - |
| | Mollusca | 60.62 | 52.38 | 85.1 |
| **Mollusca** | Bivalvia | 66.11 | 67.5 | 99.63 |
| | Gastropoda | 31.94 | 32.36 | 0.37 |
| | Scaphopoda | 1.83 | - | - |
| **Bivalvia** | *Cerastoderma edule* | 19.19 | - | - |
| | *Cerastoderma glaucum* | - | - | 18.25 |
| | *Polititapes aureus* | 18.94 | 30.16 | - |
| | ***R. decussatus*** | **3.65** | **11.68** | **57.03** |
| | *Solen marginatus* | 8.40 | 18.55 | 21.5 |
| | *Spisula solida* | 24.41 | 3.13 | - |
| | *Spisula subtruncata* | 6.51 | 4.93 | - |
| | *Venus verrucosa* | 17.7 | - | - |

Figure 1. Similarity analysis of sampling stations.




**Acknowledgements**
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# TRANSCRIPTOMIC PROFILING OF FOLLICULAR CELLS ACROSS THE ANNUAL REPRODUCTIVE CYCLE IN EUROPEAN SEA BASS *Dicentrarchus labrax*

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## Introduction

Reproductive control and prevention of precocious puberty are key for sustainable aquaculture. The European sea bass (*D. labrax*) is economically important in the Mediterranean, yet the molecular regulation of its oogenesis remains incompletely understood. Oocyte development involves systemic hormonal cues (FSH, LH, sex steroids) (1) and local paracrine signals within the ovarian follicle, between the oocyte and surrounding granulosa cells (2). Follicular cells support oocyte growth and maturation, and coordinate folliculogenesis through specific signaling pathways (3). This study aimed to investigate seasonal changes in gene expression in follicular cells over a full reproductive cycle to identify the molecular mechanisms driving follicular development (4)

## Materials and methods

Follicular cells were isolated throughout the annual cycle from ovaries of adult females kept under natural photoperiod and temperature at the Instituto de Acuicultura Torre de la Sal (IATS), following Crespo *et al*. (5). Ovaries were dissected, washed, and dissociated in M199:$H_2O$ medium with antibiotics. Enzymatic digestion was performed with trypsin (37 °C, 30 min), followed by filtration (100 µm), centrifugation, and resuspension in M199 Hanks medium. Follicular cells were counted using Trypan blue in a Neubauer chamber. RNA was extracted with the Maxwell® LEV simplyRNA Tissue kit, and quality was assessed with Nanodrop® and Bioanalyzer. Libraries were prepared and sequenced (Illumina NovaSeq X) by BMKgene.

## Results

A total of 67 genes involved in steroid hormone biosynthesis were identified. The highest number of differentially expressed steroidogenic genes occurred in spring/summer versus winter, with 13 and 15 DEGs (mostly upregulated), respectively. These data highlight dynamic seasonal regulation of gene expression in follicular cells, particularly in relation to steroidogenesis and follicle development.

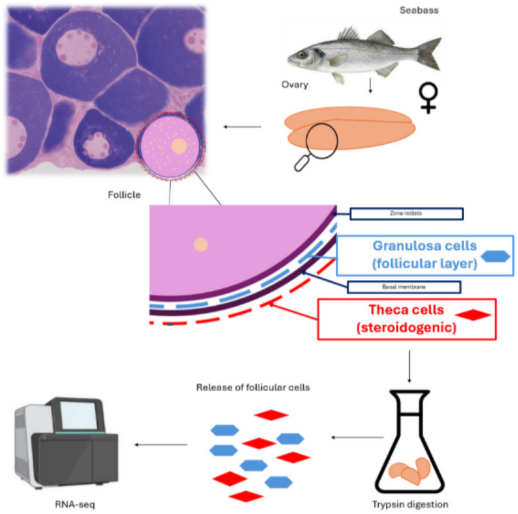


**Figure 1.** Graphic summary of the methodology used

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Transcriptomic profiles were compared using RNA-seq of follicular cells isolated from female European sea bass ovaries collected during the four seasons (summer, autumn, winter, and spring). Ovarian staging was performed by histological analysis of each ovary and classified as previtelogenic, vitelogenic, maturing/ovulating and atresic. Major transcriptional changes were observed between late winter (end of the spawning period) and summer (post-spawning), coinciding with follicular atresia and initiation of new follicle formation, so many of these differentially expressed genes are probably related to folliculogenesis. A second transcriptional shift was noted at the onset of vitelogenesis respect to the previtelogenic stage, while few changes occur during the secondary growth from initial vitellogenesis to postvitellogenesis / maturation. A total of 67 genes involved in steroid hormone biosynthesis were identified. The highest number of differentially expressed steroidogenic genes occurred in spring/summer (previtelogenesis) versus winter (spawning), with 13 and 15 differentially expressed steroidogenic genes (mostly upregulated), respectively. These data highlight dynamic seasonal regulation of gene expression in follicular cells, particularly in relation to steroidogenesis and follicle development.

**Discussion and Conclusion**

Transcriptomic analysis of follicular cells revealed two main peaks of gene expression. An increase in gene expression activity after the spawning period (late spring / summer), likely related to tissue remodeling and folliculogenesis (7). Another peak in gene expression upregulation could be observed in winter, at the beginning of the spawning phase. This last pattern aligns with previous findings on key reproductive genes (*fshr, lhr, star, cyp19a1a*) during winter in European sea bass ovaries (6). Among steroidogenic genes, *cyp11a1.2* and *hsd3b1* were consistently upregulated across seasons. In conclusion, these results demonstrate that follicular cell transcriptional activity in *D. labrax* is seasonally regulated, contributing to the understanding of ovarian development and informing improved reproductive strategies in aquaculture.

**Funding**

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# THE DETECTION AND INTAKE OF INERT MICRODIETS BY LARVAL FISH IS PREDOMINATED BY SENSORY STIMULI RATHER THAN VISUAL STIMULI: A PROOF-OF-CONCEPT STUDY

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**Introduction**
Early-stage fish larvae are widely assumed to rely primarily on visual cues such as contrast, movement, and shape to identify and capture prey like Artemia or rotifers. This perception has historically limited the use of inert microdiets in early feeding regimes. However, fish larvae possess multiple sensory modalities. In this proof-of-concept study, we explored whether marine larvae are able to detect and ingest inert feed pellets lacking conventional visual triggers.

**Methods**
We conducted exploratory trials with larvae of D. Labrax, S. Aurata, Pseudochromis spp. AquaSnow inert microdiet particles with low visual contrast and no movement were introduced into controlled tanks. Behavioural responses were recorded on video, with attention to swimming patterns, feed detection, and ingestion. No formal statistical analysis was conducted; the aim was to qualitatively assess whether larvae would actively seek out and consume the inert particles.

**Results**
Larvae across all tested species were observed to actively approach, chase, and ingest microdiet particles. This behaviour occurred consistently despite the absence of visually stimulating cues such as movement. The responses suggest that sensory modalities in addition to vision—potentially chemical (olfactory) cues—may play a role in early-stage feed detection.

**Discussion**
Our findings challenge the prevailing assumption that visual cues are essential for initiating feeding behaviour in fish larvae. While preliminary, the observations support the idea that odor or chemical leaching from microdiets may help trigger feeding responses in marine larvae. If confirmed, this opens new possibilities for microdiet formulation, particularly in enhancing palatability through targeted chemoattractants.

**Conclusion**
This exploratory study provides early evidence that marine fish larvae can detect and consume inert microdiets even in the absence of strong visual stimuli. Further research is needed to understand the specific sensory mechanisms involved, but these results point to the importance of considering olfactory cues in larval feed design.

# ACUSTO-OPTIC INTEGRATED SYSTEM FOR BLUEFIN TUNA *Thunnus thynnus* BIOMETRICS

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**Introduction**

We present preliminary results of the integration of a stereocamera and a high frequency sweeping echosounder designed to obtain biometric measurements of free swimming bluefin tuna (*Thunnus thynnus*). The stereovideo recordings can be analysed in a full automated way with the software AUTOTUNA (R) to obtain length and height of tuna. The echograms are inspected for the fishes located in the axis of the system by the 3D stereo measurement, in order to extract their lateral dimensions from echo characteristics. The three obtained fish dimensions (length, height and width) can improve the biomass estimation through multidimensional biometric relationships.

**Materials and methods**

We have integrated a S500 multipurpose single beam echosounder produced by Cerulean and a stereoscopic videocamera built by Chrome Motion Electronics (https://www.chromemotion.es/). The S500 implements chirped modulation, which sends a varying frequency ultrasonic pulse. The advantages of chirp include better signal to noise ratio, and much higher range resolution than non-chirp devices. The S500 can report a simple distance measurement or can provide raw signal strength data profiles for advanced analysis (https://ceruleansonar.com/product/sounder-s500/).

The S500 uses a high frequency 500kHz transducer giving about 5° beam width and it has a measurement range of over 100m that exceeds sufficiently the measuring range of the stereocameras, limited by visibility and precision up to 10 m. It includes three communication interfaces: Ethernet (100Mb/sec), USB (12Mb/sec), and 3.3-5.0V serial (115kbaud). The echosounder transducer is placed in the middle of the pair of stereocameras defining with its acoustical beam, the synchronised acousto-optic observation axis (Figure1). A Python based software has been developed to get the raw signal data profile of the S500.

The stereo video recordings can be analysed in a full automated way with the software AUTOTUNA (R) to obtain length and height of tuna. The echograms are inspected for the fishes located in the axis of the system by the 3D stereo measurement, in order to extract their lateral dimensions from echo characteristics. The three obtained fish dimensions (length, height and width) can improve the biomass estimation through multidimensional biometric relationships.

**Results**

The system has been tested at the ICAR-IEO and it is currently recording periodically the growing of second year individuals. Figure 2 shows the histogram of 273 length measurements automatically obtained in a 19 minute recording, and an example of corresponding echogram ping envelope and image recording.

We are developing criteria for fish width measurement from the temporal recording of the central ping in each fish trace in the echogram, using calibrated targets.

*(Continued on next page)*



Figure 1. Left: Stereoscopic videocamera and synchronised S500 echosounder developed by the UPV and Chrome Motion Electronics. Right: system deployed at the ICAR tanks; the stereo pair is set vertically for the horizontally swimming tuna.

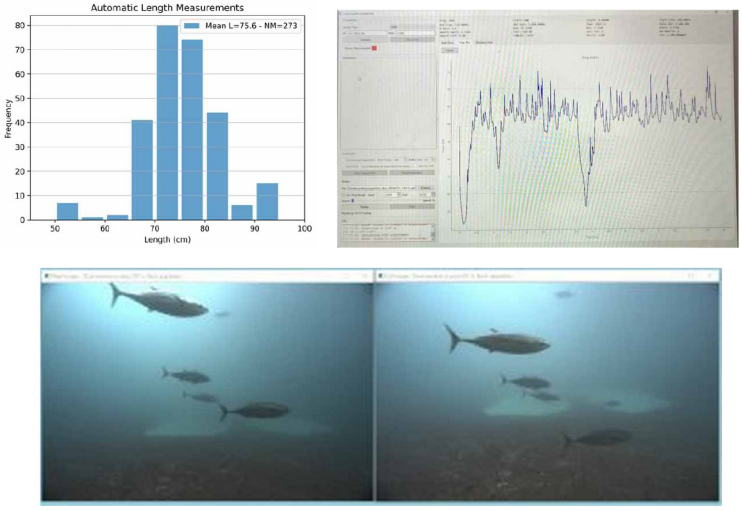


Figure 2. Upper panel left: Stereoscopic videocamera measurements of BFT furcal length distribution. Upper panel right: example of temporal characteristics of a ping of the S500 echogram, showing fish echoes at 1 m and 3.8 m from the transducer. Lower panel: stereoscopic pair of images.

## Discussion and conclusion

We have developed an integrated hardware and software solution for the synchronised use of a stereoscopic camera and a chirped echosounder for the obtention of biometric information of free-swimming tuna in tanks, cages or transfers in purse seiner operations. The system offers the possibility of obtaining 3D information of tuna shape to adjust biometric models for weight estimation (Puig *et al.* 2018).

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## Acknowledgments

The authors would like to thank the company Balfegó Tuna SL and Chrome Mortion Electronics for the contributions to the development of AUTOTUNA ® and the integrated hardware prototype respectively. This study is both part of the UPV's ACTTHUN project PID2021-127426OB-C21 financed by the Spanish AEI, and the ThinkInAzul programme, supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by the Regional Community of Murcia - Fundación Seneca (Principal Investigator: F. de la Gándara), and developed in the ICTS-ICAR, www.icar.ieo.es.

# AN INTEGRATED AUTONOMOUS SYSTEM FOR ACTIVE AND PASSIVE ACOUSTIC MONITORING OF A FLOATING SEA CAGE

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## Introduction

Over the past decades, global consumption of aquatic animal products has grown at a faster pace than the world's population. While capture fisheries have largely stabilized, aquaculture has emerged as the fastest-growing food production sector, representing more than half of global aquatic animal production since 2022. In the Mediterranean, marine aquaculture has expanded steadily, becoming a key economic sector and research field. Nevertheless, the sector faces challenges related to environmental sustainability, animal welfare, and disease control under intensive production. Advances in technology are critical to achieving the 'blue transformation' proposed by the FAO, with cost-effective monitoring systems playing a central role in sustainable management.

## Materials and Methods

We developed a stand-alone acoustic monitoring system integrating active and passive sensors for installation on floating aquaculture cages. The system comprises four modules: a solar-powered energy module with $LiFePO_4$ battery storage and MPPT charge control, a communication module providing remote access via a 4G router and TeamViewer for real-time configuration and data visualization, an acquisition unit equipped with a sound card, a mini pc (Linux or Windows-based) and a echosunder (Kongsberg WBT Mini, split-beam, 120 kHz; and Zunibal ZSR, single-beam, 120 kHz) two acoustic sensor a an Aquarian H2a hydrophone and a 120 kHz echosounder transducer (single-beam or split beam depending the chosen echosounder) that can be seen in Fgure 1 (a,b, c, d).

The hydrophone system records continuous or scheduled soundscape data using Linux or Windows-based acquisition software, while the echosounders acquire fish biomass, growth, and behaviour data. Calibration procedures were performed for both hydrophone and echosounders following standard target and reference hydrophone methods.

## Results

Hydrophone calibration in a controlled tank environment showed frequency-dependent sensitivity, with an increasing trend between 125 Hz and 4 kHz, followed by a decrease at higher frequencies. Echosounder calibration confirmed correct target strength measurements for both devices, with the scientific unit offering higher resolution but at the cost of greater storage requirements.

Field tests were conducted in a 5 m diameter fish cage located at Gandia harbour. Echograms from both echosounders showed clear detection of targets at multiple depths, validating system performance. The Kongsberg WBT Mini produced higher-definition data, while the Zunibal ZSR provided adequate quality with reduced data storage needs.

## Discussion and Conclusions

The proposed system demonstrated the feasibility of integrating low-cost active and passive acoustic monitoring in a fully autonomous platform for aquaculture. The modular design enables flexible sensor integration, while solar power and remote communication enhance operational autonomy. Preliminary results confirm that both high-end and commercial echosounders can provide useful data for fish and feeding monitoring (Soliveres et al 2017, Llorens et al. 2021) , with trade-offs between resolution and storage requirements. This approach represents a step toward cost-effective, scalable monitoring solutions for sustainable aquaculture.

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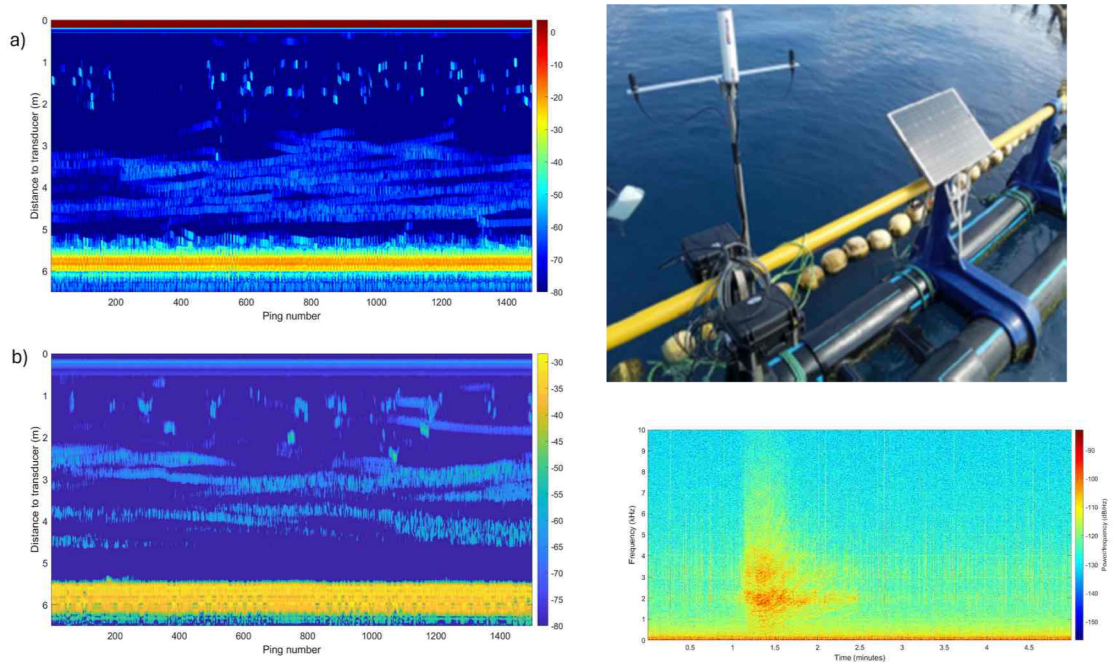


Fig. 1 - a) Echogram obtained from Kongsberg WBT Mini. b) Echogram obtained from Zunibal ZSR. c) Installation in cage rim. d) Spectrogram of a passing ship.

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# COMPARATIVE IMMUNE ASSESSMENT IN SKIN MUCUS AND HEMOLYMPH OF THE CUTTLEFISH *Sepia officinalis*.

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**Introduction**

The immune system of *Sepia officinalis* is primarily innate, relying on various cellular and humoral components to defend against pathogens (Benois et al., 2020). The main immune cells are haemocytes, which are involved in pathogen response and detoxification. The white body (WB) in cuttlefish plays a crucial role in haematopoiesis, producing haemocytes that are essential for immune responses. Additionally, the cuttlefish's haemolymph and mucus contain various immune-related enzymes and proteins that contribute to its defence mechanisms (Le Pabic et al., 2014). While the innate immune system of *Sepia officinalis* is well-adapted to its environment, it lacks the adaptive immune components found in vertebrates (Beuerlein et al., 2002). This reliance on innate mechanisms highlights the evolutionary divergence in immune strategies between invertebrates and vertebrates. The study of cephalopod immune systems, such as that of *Sepia officinalis*, provides valuable insights into the evolution of immune functions and the potential for discovering novel immune-related molecules.

**Material and Methods**

Eight male cuttlefish specimens were kept in the facilities of the marine resources centre at the IMIDA (Murcia, Spain). The study animals were fed at 1.5% of body weight daily for 1 month. Samples of skin mucus and haemolymph were obtained from each specimen. Different immune-related parameters were evaluated in the samples. The total content of protein was evaluated in both serum and skin mucus (Bradford, M., 1976), as well the protease and antiprotease activities were determined (Ross N.W. et al., 2000). Besides, the number of haemocytes was recounted and the hemocyanin concentration in the haemolymph was evaluated.

**Results and discussion**

Immune parameters were successfully quantified in both skin mucus and haemolymph samples of *Sepia officinalis*, allowing for a preliminary characterization and standardization of baseline immune profiles in this species.

The concentration of proteins in haemolymph was more than fivefold greater than that quantified in skin mucus, which appears to be consistent with the composition of haemolymph. The haemolymph functions as a medium for the conveyance of haemocytes, nutrients, gases, and metabolic byproducts, thereby clarifying the difference with the skin mucus, which solely comprises various molecules predominantly associated with immune-related functions (Castillo M.G. et al., 2015). The protease activity in haemolymph is virtually negligible; however, in skin mucus, it constitutes approximately 7% of protease activity. Correspondingly, the antiprotease activity in skin mucus significantly surpasses that observed in haemolymph, nearly doubling it (29% in the haemolymph and 46% in the skin mucus).

Statistical analysis revealed significant differences between both tissues ($p < 0.05$) for protein concentration, protease and antiprotease activities among others, suggested functional compartmentalization of immune responses in *S. officinalis*. These results represent an integrated reference values for humoral and cellular immunity in cuttlefish, providing a foundation for future studies in cephalopod health monitoring and aquaculture applications.

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**Acknowledgements**

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# DIETARY SUPPLEMENTATION OF SILKWORM *Bombyx mori* CHRYSALIS IN GILTHEAD SEABREAM (*Sparus aurata L.)*: EVALUATING ITS EFFECTS ON STRESS RESPONSE.

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## Introduction

The gilthead seabream (*Sparus aurata*) is a significant species in Mediterranean aquaculture, and its production is often challenged by various stressors that can impact fish welfare and productivity. Stress in aquaculture can arise from environmental factors, handling, and parasitic infections, leading to physiological and metabolic changes in the fish. Acute handling stress has been shown to increase blood glucose and lactate levels, as well as alter haematological profiles, indicating a physiological stress response that can affect fish welfare and product quality (Fazio et al., 2015). The use of silkworm pupae as an alternative protein source in aquaculture is being explored to replace traditional fishmeal, which is costly and often sourced from outside regions. Sericin administration has been shown to decrease serum corticosterone levels and enhance antioxidant defences in the hippocampus, reducing apoptosis and heat-shock protein levels (Mahmoudi et al., 2021). These effects indicate that sericin can modulate stress responses at the molecular level, potentially benefiting fish under stress. The integration of sericin into aquaculture practices could help manage stress in fish, particularly during critical stages such as larval development, where sensitivity to stress is higher (Jawad, 2021). By reducing stress, sericin could improve growth rates, health, and survival rates in aquaculture systems.

## Material and Methods

Forty-four specimens from gilthead seabream *Sparus aurata* were randomly disposed into eight RAS tanks. The daily feeding rate was adjusted to 3% and introduced two times per day. The animals fed for 30 days with the following diets: a control diet and supplemented diet with 1% of silkworm chrysalis (D1). Besides, the half fish from each diet group were subjected to chronic stress, according to a previously described protocol (Cámara-Ruiz, M. et al 2022). Briefly, stressed fish were subjected to 1 min air exposure twice a week for 4 weeks. Fish samples of skin mucus and blood were obtained after 15 and 30 days of feeding, six sample were randomly selected from each aquarium. Immuno-related parameters were measured in the mucus and serum (protein levels, protease and antiprotease activities). All the samples were analysed per triplicate.

## Results and discussion

At 15 days, our results indicated that stressed animals showed a slightly lower weight gain (WG) and specific growth rate (SGR) than the animals fed the control diet. The fish fed D1 showed similar WG and SGR to the control animals. However, those animals fed D1+stress showed a significant increase in WG and SGR with respect to stressed fish. At 30 days, stressed animals showed a significant decrease on WG and SGR with respect to control groups. However, no differences with control group were observed on WG and SGR from animals fed the D1 or D1+stress with respect to control group. Supplementing *Bombyx mori* pupae appears to reduce the negative effects of stress on growth, which is consistent with reported for other species, where protein substitutions from silkworm pupae meal of (10-60%) have been carried out with good results without compromising the health of the fish (Nazerath Nisha, S. et al., 2014; Shakoori, M. et al., 2016).

Protein, protease, and antiprotease activities were assessed in skin mucus and serum. No significant differences in protein levels were detected in mucus or serum at 15 days, nor in serum from stressed fish at 30 days, consistent with previous findings (Cámara-Ruiz et al., 2022). However, fish fed the D1 diet showed reduced serum protein at 30 days compared to controls, suggesting an effect on mucus immunity despite no weight loss. In skin mucus, stressed fish fed D1 also exhibited a significant protein decrease.

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At 15 days, no differences in mucus antiprotease activity were observed among groups. By 30 days, D1-fed fish showed higher mucus antiprotease levels than controls, with stressed D1-fed fish exhibiting the highest values. This suggests D1 enhances mucus defence in both stressed and non-stressed fish, consistent with reports that Bombyx mori pupae protein supports fish health (Shakoori et al., 2016). Serum antiprotease activity was also elevated in all groups compared to controls, indicating responses to both diet and stress, with stressed D1-fed fish showing the greatest improvement.

For protease activity, serum levels increased at 15 days with D1 but returned to baseline at 30 days. In mucus, D1-fed fish showed decreased activity at 15 days followed by a rebound at 30 days. Stressed fish were unaffected, except when fed D1, which significantly increased mucus protease, suggesting enhanced immune defence.

Our findings demonstrate that a 30-day dietary supplementation with *Bombyx mori* pupae significantly enhanced growth performance and non-specific immune defenses in gilthead seabream (*Sparus aurata*) subjected to chronic stress. These results highlight the potential of *B. mori* pupae as a functional dietary ingredient (even at 1%) to improve resilience and overall health in aquaculture species under challenging farming conditions.

**Acknowledgements**
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# TRANSCRIPTOMIC PROFILING OF GILTHEAD SEA BREAM (*Sparus aurata*) LIVER REVEALS DIFFERENTIAL GENE EXPRESSION IN RESPONSE TO FISH OIL-ENRICHED DIETS

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## Introduction

Gilthead sea bream (*Sparus aurata*) is extensively cultivated in the Mediterranean due to its economic significance and its adaptability to diverse aquaculture systems. Despite major advances, challenges remain in nutritional management and prevention of metabolic diseases in sea bream farming (Hallerman et al., 2022). The liver plays a crucial role in fish metabolism by regulating lipid mobilization under nutritional stress. Diet composition, particularly fatty acid type and quantity, impacts liver function, energy balance, and disease resistance. High lipid diets may induce to hepatic steatosis, which in turn impairs liver function and triggers inflammation, fibrosis, and metabolic disorders. In sea bream, steatosis negatively influences growth, feed efficiency, and infection resistance, potentially leading to increased mortality and decreased product quality, thereby affecting aquaculture profitability (Oliva-Teles, 2012; Turchini et al., 2009). Understanding differential gene expression is essential to elucidate how lipid-rich diets disrupt lipid homeostasis, and promote fatty liver. Integrating transcriptomic methodologies with novel nutritional approaches is vital for improving fish health and fostering sustainability in aquaculture (Asaoka et al., 2014; Costa-Silva et al., 2017). Consequently, the objective of this study was to assess hepatic gene expression changes in gilthead seabream on a fish fed a fish-oil-enriched diet.

## Material and Methods

Juvenile sea bream (*S. aurata*) with an average weight of 10-14 g were kept in the facilities of the experimental animal service (SEA) at the University of Murcia (UMU). The study animals were fed at 1.5% of body weight daily for 3 months with the following diets (two replicates): i) standard diet (Skretting, Control Group), ii) diet enriched with 10% fish oil (Fish oil Group). At the end of the experiment, the fish were sacrificed (n = 6 per group), the liver was obtained and frozen in liquid nitrogen. Total RNA was extracted from the liver and its integrity was verified, analysing its profile with a bioanalyser. cDNA library construction was carried out using the Illumina Stranded mRNA Prep' protocol. The libraries were sequenced using a 2 x 100 bp paired-end sequencing strategy, with a minimum sequencing depth of 25 million pairs of reads (50 million individual reads) per sample using the NextSeq 2000 sequencer (Illumina, USA). In silico differential expression analysis was carried out using Rstudio 4.3.0 software.

## Results and discussion

A total of 385,292,118 reads were obtained from 12 cDNA libraries (380,669,063 clean reads). From these clean reads, expression counts were obtained for a total of 32,008 genes, according to the available genomic annotation. So, 463 significantly differentially expressed genes were identified, of which 158 were found to be up-regulated and 305 down-regulated.

Taken together, our data confirm the existence of a specific transcriptional response associated with fish oil consumption. Up-regulated genes were clustered in categories linked to transport activities, hydrolase and oxidoreductase enzymes, whereas genes involved in triglyceride-lipasa activity were prominently down-regulated. Enrichment analysis highlighted oxygen binding, haemoglobin binding and olfactory receptor binding together with RNA-binding, ligase and glycosyl-transferase activities, suggesting a complex physiological response to treatment. In contrast, functions linked to the transport of organic ions and anions were suppressed. These results highlight the modulation of key functions in cell metabolism and signalling in response to fish oil treatment.

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The Peroxisome Proliferator-Activated Receptor (PPAR) signalling pathway was significantly over-represented among the differentially expressed genes., coherently linking the transcriptional response to lipid metabolism, energy balance and inflammation. This is consistent with a possible physiological effect related to the treatment administered, especially if associated with nutritional intervention or lipophilic compounds such as fatty acids (Antonopoulou et al., 2017). The most prominent pathway was 'Ribosome biogenesis in eukaryotes', ($p<0.0029$) and a Gene Ratio higher than 0.46. The 'One carbon pool by folate' pathway exhibited significance, albeit to a lesser extent. These findings suggest a potential reconfiguration of ribosomal components and basic cellular metabolism, possibly linked to a specific physiological response induced by the high fatty acid diet employed in this study. (Colombo, 2020).

Overall, these findings elucidate the molecular mechanisms by which high fatty acid diets influence liver health in gilthead seabream. The identification of specific genes and pathways associated with dietary response lays a foundation for optimizing dietary formulations in Mediterranean aquaculture, enhancing the health, welfare, and production efficiency of this important species.

**Acknowledgements**

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# MULTIOMICS OF EUROPEAN SEABASS (*Dicentrarchus labrax*) EXPOSED TO ACUTE STRESS BY TRANSPORT

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**Background**
Unravelling new knowledge on biomolecular response to acute stress in farmed fish is essential for better monitoring, improve animal welfare and health, and reduce mortality. This study aimed to evaluate physiologic and metabolic effects of acute stress induced by transport in European seabass (*Dicentrarchus labrax*). Trials were conducted at University of Cádiz facilities.

**Methods**
After acclimatisation, fish were divided in four groups of fish. One of the groups was immediately sampled to serve as a non-stressed initial control. Two groups of fish were transferred each to three oxygen saturated containers and transported by a transport van for 4 hours. Upon arrival, half were immediately sampled while the remaining fish were put in the original system and sampled at 24h post-arrival as recovery group (n=12 per sampling time). A fourth group was kept under control conditions and sampled at 24h.

RNA-seq
Total RNA was extracted from skin tissue and pooled (n=4 samples per treatment) for RNAseq on an Illumina sequencer. Raw sequence reads were trimmed and filtered using fastp, to remove adapter sequences and low-quality reads. Transcripts were quantified using the pseudoalignment method implemented in kallisto. Differentially expressed genes (DEGs) between the different treatments and the control samples were determined using DESEQ2.

Proteomics
Skin mucus samples from the same fish were processed for proteomic analysis following the solid-phase-enhanced sample preparation protocol and digested with Trypsin/LysC enzymes (Osório et al., 2021). Proteins were identified and quantified by nanoLC-MS. Raw data was processed in Proteome Discoverer v.3.0.1.27 (Thermo Scientific) and searched against the UniProt database for Eupercaria (1.1 million proteins). Protein label-free quantitation was performed with the Minora feature detector node at the processing step. Raw data hits were filtered using coverage above 30%, unique peptides above 2 and a SEQUEST HT score greater than 100.

**Results**
Data showed different genes regulated during transport induced acute stress. In addition, the European seabass transcriptome returned to base level expression by 24h (fig.1), in contrast with the proteome (fig.2). This indicates that while the effect of the stress may no longer be detectable at the gene transcript level, the physiological response continued, as peptide levels were still modulated. Further studies are needed to determine when protein levels return to base level. Gene Ontology (GO) terms upregulated in the transcriptome during stress include genes implicated in circadian rhythm (GO:0032922, GO:0007623, GO:0048511), negative regulation of cellular processes (GO:0043066, GO:0043069, GO:0048523, GO:0048519) and downregulated were genes related to nervous system development (GO:0007420, GO:0060322, GO:0007417), DNA-binding (GO:0043565, GO:0003700) and transcription (GO:0140110, GO:1903506, GO:0006355).

At the proteomic level, differentially expressed proteins are associated with GO terms regarding the activation of the immune response (GO:0002253, GO:0050778, GO:006959, GO:0002252) and wound healing/coagulation (GO:0030195, GO:0061045). These results elucidate the cascade of events that occur after acute stress in seabass. Further analysis we are working on with other species (trout and salmon), other types of stress and microRNAs will enable us to generalize these results and find common responses between species and have a more detailed view of all the processes affecting them.

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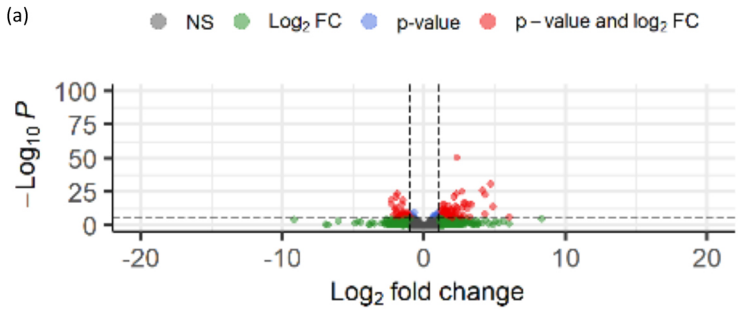
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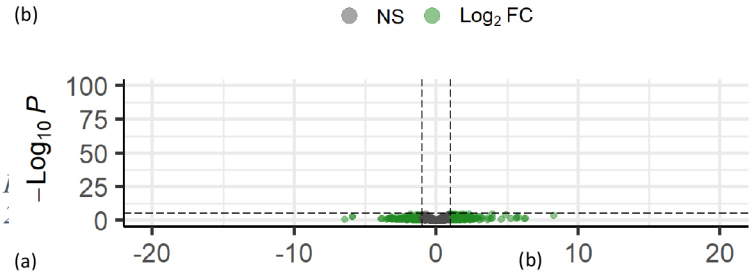
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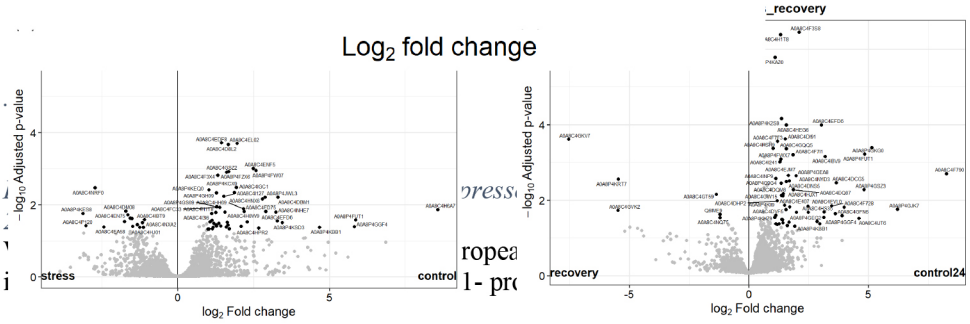
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# ENHANCING OYSTER RESILIENCE FOR BIOREMEDIATION: DIETARY INFLUENCE OF MAR MENOR MICROALGAE ON ANTIOXIDANT AND IMMUNE FUNCTIONS

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**Introduction**

The Mar Menor lagoon, located in the south-east of the Iberian Peninsula, is one of the most unique coastal ecosystems in the western Mediterranean. However, in recent decades it has suffered progressive environmental degradation, mainly as a result of the intensification of agriculture in its river basin, unplanned urban development and wastewater discharges (Paredes et al., 2021; Velasco et al., 2022). These factors have favored eutrophication processes, characterized by high nutrient concentrations, phytoplankton proliferation, loss of macrophytes, episodes of anoxia and occasional ecosystem collapses, such as the one that occurred in 2016 (García-Ayllón, 2018). In response to this situation, nature-based solutions have been proposed to promote the resilience of the system and restore some of its ecological functions. Among these, the use of filter-feeding bivalves as a bioremediation tool stands out. *Ostrea edulis*, the European flat oyster, is a species with a high capacity to filter and retain particles, organic matter and phytoplankton (Pogoda et al., 2019; Bricker et al., 2020), which can contribute to improving water quality and promoting the recovery of degraded habitats. Its reintroduction or experimental cultivation in the Mar Menor could represent a complementary strategy for the functional restoration of the ecosystem. This study aims to investigate the influence of different dietary microalgae on the antioxidant activity and immune parameters in European flat oysters, with a focus on three key physiological compartments: hemocytes (cellular immunity), hemolymph and intervalvar fluid (humoral immunity). By exposing oysters to controlled microalgae diets simulating the Mar Menor ecosystem, we evaluate how species-specific algal blends modulate oxidative stress and immune defenses across these interconnected systems.

**Materials and methods**

An *in vivo* experimental trial was conducted under different dietary conditions simulating real scenarios in the Mar Menor over a 30-day period, using adult oysters (Ostrea edulis). The study aimed to evaluate their subsequent immune response and antioxidant activity. The oysters were randomly divided into three groups: a control group (fed with commercial gel phytoplankton containing 33.3% *Isochrysis* T-Iso, 31% *Nannochloropsis*, 18% *Tetraselmis*, and 18% *Phaeodactylum*) and two experimental groups fed with different microalgae cocktails prepared from selected Mar Menor strains to replicate the ecosystem's environmental conditions. The first cocktail (Cocktail 1) contained *Mayamea* sp., while the second (Cocktail 2) consisted of a mixture of *Mayamea* sp. and *Erythrolobus* sp. The microalgae were cultured under controlled conditions before being used as oyster feed.

Samples were taken from oysters on days 0, 15 and 30 of the trial. The hemolymph was extracted by puncturing the abductor muscle, and the intervalvar fluid was also obtained. The samples were centrifuged at 4°C to separate the cells and the supernatants, which were stored at -20°C until analysis.

Total antioxidant activity was determined in the microalgae used in the assay as well as in the cells obtained from hemolymph and intervalvar fluid in order to characterize the physiological responses induced by exposure to microalgae cocktails. In the supernatants of both hemolymph and interstitial fluid, soluble proteins were quantified using the Bradford colorimetric method, with bovine serum albumin (BSA) as the standard. Enzyme activities were analyzed using spectrophotometric assays: antiprotease activity was measured through trypsin inhibition; lysozyme activity was measured through the lysis of the bacterial substrate *Micrococcus lysodeikticus*; and peroxidase activity was measured through 3,3',5,5'-Tetramethylbenzidine oxidation. In addition, total antioxidant capacity was evaluated.

The experimental design allowed for analysis of temporal variation within each treatment and analysis of the differential effects between diets, as well as exploration of functional differences in immune responses between hemolymph cells and interstitial fluid cells.

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Statistical treatment of the data was performed using multifactorial analysis of variance (ANOVA), followed by post hoc tests (Tukey HSD), with a statistical significance level of $p < 0.05$. The analyses were performed using R software (v4.3.0).

**Results and discussion**

In terms of antioxidant activity, the hemolymph exhibited a consistent response across various treatments and over time. In contrast, the intervalvar fluid in the control group demonstrated a twofold increase in antioxidant activity between days 15 and 30, suggesting a potential accumulation of oxidative stress. Groups that were administered microalgae displayed stable or reduced levels. Furthermore, analysis of the microalgae utilized revealed significant differences in antioxidant activity within both the lysate fraction and the supernatant, indicating that each cocktail contributes uniquely to the redox profile and may differentially affect the response of oysters. Concerning immune parameters, antiprotease activity in the hemolymph remained stable, with no significant variations attributable to treatment or time. Conversely, the intervalvar fluid showed a treatment-dependent decrease, particularly in the group fed cocktail 2, where the reduction was progressive over time. Regarding lysozyme activity, hemolymph levels remained consistent on both sampling days for the control group and cocktail 1, with no significant differences observed. Only cocktail 2 exhibited a notable peak on day 15, followed by a decline on day 30. In the intervalvar fluid, the pattern was reversed: values were low and similar on day 15, but by day 30, an immune activation was evident, especially in the cocktail 1 group, with a significant increase; cocktail 2 and the control also increased, though without significant differences. Peroxidase activity in hemolymph showed an upward trend on day 30 across all groups, reaching statistical significance in the control and cocktail 2. In the intervalvar fluid, the response was more varied: the control group decreased significantly, while cocktail 2 exhibited a marked increase with significant differences. This study demonstrates that dietary microalgae from the Mar Menor lagoon can significantly modulate antioxidant activity and immune responses in oysters across different physiological compartments. The hemolymph showed relatively stable antioxidant activity, while the intervalvar fluid exhibited more dynamic responses. The microalgae cocktails, especially cocktail 2 induced distinct patterns of immune activation, notably in lysozyme and peroxidase activities. These findings suggest that carefully selected microalgae diets could potentially enhance the oysters' physiological resilience, which may be beneficial for bioremediation efforts in the Mar Menor ecosystem. However, the complex and sometimes contrasting responses observed between hemolymph and intervalvar fluid underscore the need for comprehensive evaluation of multiple parameters when assessing bivalve health and function in restoration contexts. Further research is warranted to optimize microalgae formulations and to investigate long-term effects on oyster populations and ecosystem services in the Mar Menor lagoon.

**Acknowledgements**

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# ULTRASOUND-ASSISTED EXTRACTION OF COLLAGEN FROM FISH SKIN: A TIME-EFFICIENT STRATEGY FOR MARINE BY-PRODUCT VALORIZATION

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## Introduction

The growth of the global population, combined with shifts in dietary patterns, has elevated food security to a matter of critical importance. Within this context, seafood production has experienced significant growth over recent decades, exceeding 180 million tons in 2022 (FAO, 2024). The increase in production has also resulted in a higher volume of by-products, with fish by-products estimated to exceed 20 million tonnes (Yuan et al., 2024). Efforts to reduce the waste of these by-products have led to their use in the production of fishmeal and fish oil. Beyond their use in animal feed production, this biomass contains a significant range of bioactive compounds, offering opportunities to expand the potential applications of these by-products. Collagen falls into this category, with broad applications across food, cosmetic, and medical industries. Collagen serves as a key structural protein in animal tissues and it is particularly abundant in fish by-products, such as skin, scales, and bones. Conventional collagen extraction methods often suffer from inefficiencies, including long pretreatment and extraction times, low extraction yields and the reliance on enzymes (Laasri et al., 2023). Recent years, environmentally friendly techniques have been developed to significantly enhance extraction efficiency. Among these, ultrasound-assisted extraction (UAE) has emerged as a powerful approach, through cavitation, it can significantly improve collagen extraction times and yields. This study aimed to optimize the ultrasound-assisted extraction of collagen from the skin of *Pollachius virens*.

## Materials and methods

The skin of *P. virens* was minced and 10 g of tissue were mixed with 150 mL of 0.1 M NaOH to remove non-collagenous proteins. Two amplitudes (30% and 50%) of sonication were evaluated. Following sonication, the mixtures were incubated at 4 ºC for 24 hours. The soluble protein content in the supernatant was quantified, and the molecular weight distribution of the extracted proteins was analyzed by SDS-PAGE.

Collagen ultrasound-assisted extraction was then performed on 10 g of pretreated skins with 100 mL of 0.5 M acetic acid at 4 ºC. Both conventional acid extraction (AE) and ultrasound-assisted extraction at three amplitudes (40%A, 60%A and 80%A) were assessed. Re-extraction of collagen was also evaluated (80%A*2), the solid residue obtained after the initial extraction at 80% was subjected to a second extraction with 100 mL of fresh acetic acid solution under the same conditions for 10 minutes. The collagen yield was calculated based on hydroxyproline content in the extraction supernatant following the method described by Cissell et al. (2017). The molecular weight distribution of the extracted collagen was analyzed with SDS-PAGE.

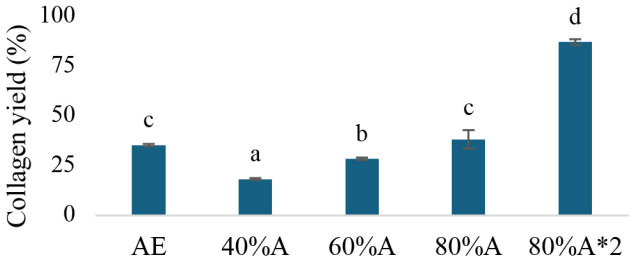


**Figure 1.** Extraction yield of collagen performed with conventional extraction for 48 hours (AE) and ultrasound-assisted extraction (40-80%A and 80%A*2). Different letters indicate significant differences ($P<0.05$).

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**Results and discussion**

A positive correlation was observed between the amplitude of sonication and the yield of protein extraction during both the pretreatment and collagen extraction stages. During the alkaline pretreatment, sonication at 50% amplitude for 20 minutes achieved a protein yield of 7.7 g per 100 g of dry skin. Notably, extending the treatment by maceration for an additional 24 hours did not further increase the protein concentration, unlike other amplitude and time combinations evaluated.

The molecular weight profile of the proteins extracted indicated that collagen was not co-extracted during this step, suggesting that it remained intact. This approach significantly accelerates the removal of non-collagenous proteins, reducing the required processing time from 6–24 hours to merely 20 minutes without compromising the structural integrity of the collagen present in the skin.

For the subsequent collagen extraction, the effects of varying amplitude were systematically assessed (Figure 1).

Collagen extraction using ultrasound at 80% amplitude for 20 minutes resulted in a collagen recovery of 38.3%, comparable to the 35.4% obtained through conventional method after 48 hours of extraction. Re-extraction of the solid residue from the initial extraction further increased total final yield to 87.3%, the highest among all tested conditions. Similar results were obtained for baltic cod (*Gadus morhua*) skin after 24 hours of extraction (Sadowska et al., 2003). The molecular weight distribution of the extracted collagen revealed the presence of protein bands corresponding to the α, β, and γ chains characteristic of type I collagen (Shoulders and Raines, 2009).

This methodology offers a efficient route for the valorization of by-products from the aquaculture and fisheries industries, enabling collagen recovery through an extraction process that can be completed in approximately one hour.

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# ANTIVIRAL ACTIVITY OF A CISTUS SPECIES AQUEOUS EXTRACT AGAINST FISH VIRUSES: A CIRCULAR ECONOMY APPROACH TO SUSTAINABLE AQUACULTURE

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**Introduction**

The growing demand for sustainable and environmentally friendly health solutions in aquaculture has intensified interest in natural bioactive compounds. Screening plant extracts from aromatic and traditional medicinal herbs offers promising opportunities for developing therapeutic agents aligned with ecological and economic sustainability. The Cistus genus, a Mediterranean shrub, is known for its high polyphenol content and secondary metabolites with potential antiviral properties (Zalegh et al. 2021).

Viral infections remain a major challenge in aquaculture, causing significant economic losses and threatening the long-term viability of the industry worldwide. While preventive measures such as vaccines are available for certain pathogens, effective antiviral treatments are still limited. In this context, natural compounds provide a valuable source of new bioactive molecules, many of which remain unexplored. Investigating their biological activity is essential to identify novel mechanisms of action and alternative therapeutic strategies (Khang et al. 2025).

This study evaluates the antiviral activity of an aqueous extract from a Mediterranean plant against a panel of viruses that are highly relevant to the aquaculture sector. By exploring the pharmacological potential of this extract, this study contributes to the valorisation of forest waste from *Cistus* species. integrating circular economy principles into sustainable aquaculture practices.

**Materials and methods**

An aqueous extract was prepared from dried plant material, which was then solubilized in dimethyl sulfoxide for testing. The antiviral activity was then assessed *in vitro* against five relevant fish viruses: spring viremia of carp virus (SVCV), viral hemorrhagic septicemia virus (VHSV), cyprinid herpesvirus 3 (CyHV-3), tilapia lake virus (TiLV), and common carp paramyxovirus (CCPV).

Each virus was incubated with host cells at virus-specific multiplicities of infection (MOIs) for two hours at the optimal infection temperature. To evaluate the antiviral activity, three experimental conditions were evauated: (i) pre-incubation of viral particles with the extract, (ii) pre-treatment of cells prior to infection and, (iii) post-infection treatment to asses effects on later replication stages. Antiviral efficacy was evaluated by quantifying the percentage of infection inhibition compared to untreated controls.

| Virus | Primary host species(es) | $IC_{50}$ (µg/ml) |
|---|---|---|
| Spring viremia of carp virus (SVCV) | Cyprinids | 33 |
| Viral hemorrhagic septicemia virus (VHSV) | Salmonids | <12 |
| Cyprinid herpesvirus 3 (CyHV-3) | Cyprinids | <12 |
| Tilapia lake virus (TiLV) | Tilapias | 12 |
| Common carp paramyxovirus (CCPV) | Cyprinids | <12 |

*(Continued on next page)*

**Results**

The aqueous extract exhibited potent antiviral activity against all of the viruses tested. Preliminary inhibitory effects were observed with VHSV, CyHV-3, TiLV and CCPV, while more robust inhibition was demonstrated for SVCV. Extended experiments with SVCV provided further insights into potential mechanisms of action. The half-maximal inhibitory concentration ($IC_{50}$) values for each virus are summarized in the table below:

**Discussion**

The aqueous extract demonstrated antiviral activity against all the fish viruses tested, although the extent and depth of the analysis varied depending on the viral model. Antiviral effects were observed when cells were treated throughout the full infection cycle for VHSV, CyHV-3, TiLV, and CCPV, suggesting that the extract can interfere with one or more stages of the viruses replication. However, further studies are needed to clarify these mechanisms.

A broader experimental design for SVCV revealed that antiviral activity was primarily observed when the extract was applied during early infection stages, either by direct interaction with viral particles or by pre-treatment of cells. No inhibitory effect was detected when the extract was added post-infection, indicating it does not affect later stages of viral replication. These results suggest that the extract acts on viral attachment, entry, or modulation of host cell susceptibility (Aoki-Utsubo et al. 2018)

Overall, these findings demonstrate the extract's multi-target antiviral potential and support the integration of botanical compounds into sustainable fish health management strategies.

**Conclusion:**

This study highlights the antiviral potential of a plant-derived aqueous extract against multiple fish viruses. The extract was particularly effective during the early stages of infection, suggesting its suitability as a preventive strategy in aquaculture. Its use aligns with circular economy principles by valorizing botanical resources for sustainable, low-impact health solutions. Further research will be essential to isolate the active compounds and support the translation of these findings into practical applications in fish farming systems.

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# MODELLING (3D) FISH ANATOMY AND PATHOLOGY AS AN INNOVATIVE TOOL: THE FIRST STEP TO CREATE A VIRTUAL MANUAL INTENDED FOR TEACHING

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**Introduction**

The study and identification of pathologies in marine species are essential for health management in aquaculture, as well as for the knowledge and conservation of marine biodiversity and promoting scientific dissemination. However, the study of this area has been limited by the need to use real biological specimens, often requiring dissection or sacrifice, which poses significant ethical, logistical, and pedagogical drawbacks. In recent years, advances in three-dimensional modelling technologies have improved the way in which knowledge is transmitted. Despite its great potential, the application of 3D modelling in the field of marine pathology remains scarce and underdeveloped in educational contexts. This highlights the need to explore and validate new visual and digital approaches that allow replacing the use of real organisms, in line with the "3Rs" principle (Reduce, Reuse and Recycle), while also improving the accessibility, understanding and dissemination of these contents.

This work arises from the desire to respond to these limitations by developing high-precision digital models that allow observing and understanding the external pathologies in sea bream. This approach is intended to contribute to the advancement of more ethical and technologically modern teaching, as well as offering a support resource for the training of future professionals in marine sciences, veterinary, and marine conservation.

**Materials and methods**

The software used for modelling was ZBrush, developed by Pixologic and currently managed by MAXON. ZBrush is a 3D modelling environment specialized in digital sculpture, which allows working with high-resolution organic models thanks to a proprietary architecture called "pixol". Pixol stores additional three-dimensional information such as depth, orientation and colour of each point on the surface. First step: the cone base is obtained, a fusiform structure with the exact dimensions of the reference model but without any type of detail (Fig.1); second step: modular body creation (eyes, mouth, operculum, fins…) (Fig. 2); third step: projection of the three-dimensional surface of the model onto a two-dimensional plane; fourth step: to represent alterations, pathologies, infections in the model.

**Results**

This methodology enables a highly realistic visual finish, resulting in an accurate base model that accurately represents the fish species (Fig. 3A). The model developed illustrate some of the typical pathologies of bream: lordosis (Fig. 3B), as an example of skeletal alterations; infection by *Tenacibaculum maritimum* (Fig. 3C), as an example of an identifiable skin infection; and the ulcerative process known as "red rash" (Fig. 3D)., a multifactorial pathology typically associated with intensive aquaculture.

To ensure the model utility, a website has been created where all of them can be viewed. The website uses the Sketchfab, an online platform that showed 3D models to be viewed interactively without requiring additional software. This results in a simple but organized web page that allows users to conveniently visualize and explore the generated models.

**Conclusions**

The developed models have a high level of anatomical precision and detail that allows them to be used in the educational and outreach sectors. They serve as visual tools for aquaculture technicians or veterinary, and students of marine biology, aquaculture, veterinary or environmental sciences, facilitating the understanding of the morphology and common diseases of this species of commercial interest. This work highlights the importance of combining scientific knowledge, technology and aesthetic sensitivity to create innovative resources that promote an education that is more respectful of living beings and more accessible to everyone.

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Figure 1. Sequential screenshots taken within the z-brush program to visualize the evolution of the model base. QR code that links to the video of the 3D modelling process carried out in ZBrush. https://www.youtube.com/watch?v=ugADveb0IL0

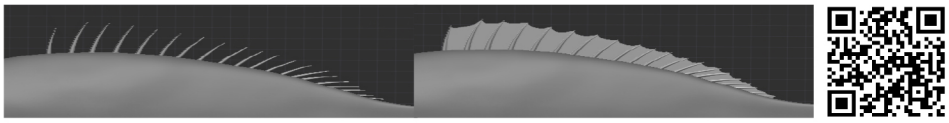
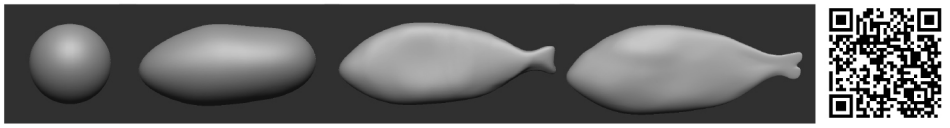


Figure 2. Screenshots taken within the z-brush program to visualize creation stages of the dorsal fin. QR code that links to the video of the 3D modelling process of the dorsal and anal fins made in ZBrush. https://youtu.be/vTken6BpQiw



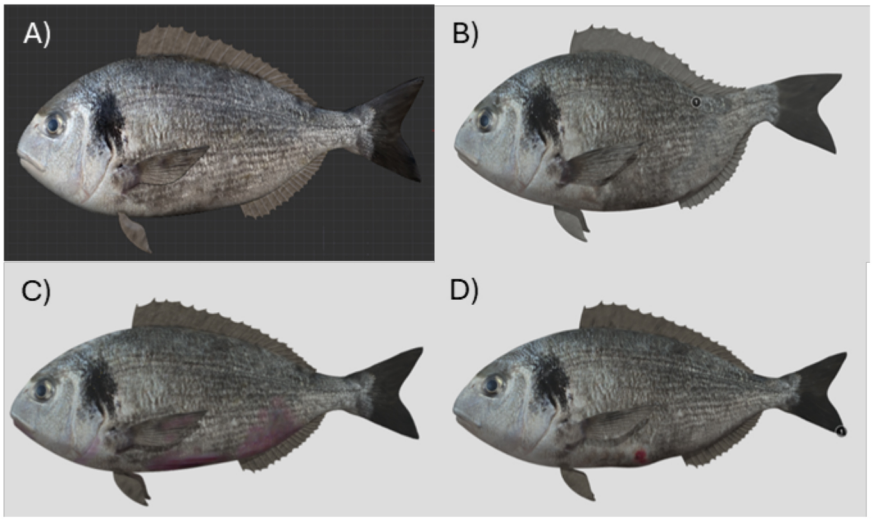



Figure 3. Screenshot taken within the Sketchfab program that allows visualize the result of the model of the sea bream (A), with lordosis (B), infected by *Tenacibaculum maritimum* (C), and showing a started "Red Rash" (D).

# VALORISATION OF AGAR BY-PRODUCTS IN EUROPEAN SEABASS (*Dicentrarchus labrax*) DIETS: A BIOCIRCULAR APPROACH TO OPTIMISE WASTE MANAGEMENT IN RECIRCULATING AQUACULTURE

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## Introduction

The aquaculture sector increasingly prioritises resource efficiency and sustainability, particularly within recirculating aquaculture systems (RAS), which can play a pivotal role in mitigating environmental impact by facilitating water reuse and reducing waste accumulation. Optimising feed formulations in RAS is essential for enhancing nutrient efficiency, with macroalgae by-products, including those obtained from agar extraction industries, representing a sustainable alternative that does not compete with terrestrial resources [1]. However, these by-products' high non-starch polysaccharide (NSP) content may hinder digestibility in carnivorous fish species, limiting their dietary inclusion. To address this challenge, nutritional strategies such as pre-treatments (e.g., chemical hydrolysis) and exogenous enzymes have been proposed to improve NSP breakdown and enhance nutrient bioavailability [2]. Despite this drawback, NSP-rich ingredients may improve RAS performance by enhancing faecal binding, resulting in larger and more compact faeces that are efficiently removed by mechanical filtration [3], ultimately lowering management costs. Thus, this study explores the potential of high inclusion of an alkaline pre-treated *Gelidium corneum* solid by-product (GBP) obtained from the agar extraction industry, both alone and in combination with exogenous enzymes, on digestibility and fish faecal physical properties, aimed at tailoring RAS aquafeed for European seabass.

## Materials and methods

Four isonitrogenous, isolipidic, and isofibrous experimental diets (CP 44%, EE 16%, crude fibre 1.7%) were formulated: one control diet and three test diets with 15% alkaline pre-treated *Gelidium corneum* by-product (GBP; NaOH 1N, 15 min autoclaving), replacing soybean and whole wheat meals, supplemented or not with 1% or 2% of a commercial thermostable NSP-degrading enzyme complex (Natugrain® TS-Feed Enzyme, BASF, Germany). All diets included 0.5% DM chromic oxide as a digestibility marker. The trial was conducted at CIIMAR (Matosinhos, Portugal) using 108 European seabass (79.9±2.4 g) randomly distributed into 12 thermo-regulated RAS tanks equipped with faecal sedimentation columns. Diets were randomly assigned to triplicate groups, fish were hand-fed to satiation twice daily, and faeces were collected before the first meal, centrifuged, and stored frozen per tank over 14 days. Tanks were cleaned 30 minutes post-feeding to remove residual waste. Apparent digestibility coefficients (ADCs) for dry matter, protein, lipid, gross energy, inorganic and organic acid detergent fibre (ADF), neutral detergent fibre (NDF), and amino acids were determined following standard protocols [4]. Fresh faecal samples from each tank were analysed for particle size distribution (fines ≤0.5 mm; medium 0.6–1.2 mm; large ≥1.2 mm) using volumetric and photometric methods [5]. Data related to ADCs and faecal physical traits were analysed by ANOVA using the PROC GLM of SAS, with diet as a fixed effect. Least square means were compared using Bonferroni's test, with a significance level set at $p<0.05$.

## Results and discussion

The ADCs of energy (84.4%, on average), dry matter (67.6%), protein (92.6%), and lipid (97.5%) were similar among control and the three test diets ($p>0.05$), aligning with reference values reported for most carnivorous fish species [6]. Inorganic and organic ADF fractions were undigestible in all dietary treatments. In contrast, the digestibility of the NDF fraction was higher in the GBP diet supplemented with 2% Natugrain® compared to the control and non-supplemented or 1% Natugrain® supplemented GBP diets (35.5% vs. 21.8% on average, $p<0.05$). These results confirm the reported beneficial effect of dietary supplementation with exoenzymes (i.e., carbohydrases) to complex polysaccharides, releasing carbohydrate oligomers and

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monomers, which reduces polymerisation and viscosity and increases bioavailability to the animal [7]. The ADCs of essential amino acids remained high across all diets, with those of lysine and methionine, key for growth and metabolism, exceeding 90%, indicating that 15% GBP inclusion did not affect their bioavailability. In addition, dietary inclusion of GBP, alone or enzyme-supplemented, enhanced the physical properties of faeces, increasing the volume (+12%) and length (+13%) of large-sized faeces (≥1.2 mm) compared to the control diet ($p<0.05$). This effect is likely attributed to fibre components of GBP, which may enhance water retention and contribute to a more compact faecal matrix, increasing gastrointestinal motility and promoting faecal aggregation [5].

**Conclusions**

Overall, 15% dietary inclusion of GBP did not affect energy, macronutrients, and amino acids digestibility, whereas supplementing the GBP-based diets with 2% of a carbohydrase enzyme complex enhanced the NDF digestibility. In addition, diets containing GBP improved faecal characteristics. Given the importance of water quality management in RAS, these findings suggest that incorporating low-cost by-products, such as GBP, into aquafeeds may optimise waste management and increase the economic sustainability of closed systems. Further studies should assess the impact of dietary GBP inclusion on fish growth, health, and water quality, while optimising aquafeed formulations for different farming conditions to enhance resource efficiency.

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# PUBERMULLET PROJECT - UNRAVELLING THE PUBERTAL SIGNALS AND GAMETOGENESIS DEVELOPMENT IN FARMED FLATHEAD GREY MULLET (*Mugil cephalus*)

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**Introduction**

The flathead grey mullet (*Mugil cephalus*) is the most commercially valuable mullet species and one of the top omnivorous marine fish in global trade. With a trophic level of 2.5, it aligns closely with molluscs and is well-suited for aquaculture due to its resilience and adaptability to various farming conditions. In 2019, global production reached 118,000 tons, with aquaculture accounting for just 6,124 tons, relying entirely on wild-caught juveniles[1]. This species is economically prized, particularly for its salted and dried roe, "*bottarga*", considered a delicacy in the Mediterranean and Asia. However, wild stocks are declining, and aquaculture is unsustainable due to the species' inability to reproduce in captivity. Flathead grey mullet do not mature or spawn in intensive culture. Research at IRTA-La Ràpita has made progress using recombinant gonadotropins (rGth) to stimulate spermatogenesis and oogenesis[2,3], but key aspects of the species' reproductive biology remain unknown. Moreover, numerous significant questions regarding the reproductive biology of the flathead grey mullet remain unanswered, and discovering their solutions will be crucial in achieving a closed cycle within aquaculture conditions. Some of these questions are: i) What are the puberty signals of the farmed flathead grey mullet? ii) What are the underlying patterns of gametogenesis prior to, during and after puberty in the flathead grey mullet? The PUBERMULLET project aims to address questions about puberty signals and gametogenesis patterns to close the life cycle in captivity. The ultimate goal is to support sustainable aquaculture of this species with minimal reliance on fish oil and fish meal.

**Material and Methods**

For this purpose, three experimental WPs will be performed in this project:

*WP1. Identification of the physiological and molecular signals when the gonad is receptive to rGths to induce puberty in the farmed flathead grey mullet juveniles.*

This WP aims to describe the putative signals in the BPG axis at different levels in the juvenile stage to reveal the onset of puberty. Steroids (E2, 11-KT and T) concentrations will be determined in blood plasma using specific antibodies by ELISA (T1.1). Brain and gonad transcriptomic profiles focusing on genes related to puberty (e.g. *kiss1r*, *gnrh2*, *gnrh3*, *leptin*, *amh*, *igf3*), gonadotropins (*fshr* and *lhr*) and their variation according to different juvenile stages will be quantified using qPCR. Histology of the gonads will be performed to observe the different evolution of the gonads, and *in situ* hybridisation will be carried out to observe the localisation of some specific genes in males and females (*fshr* and *lhr*) (T1.2). At the end of this WP, it is expected to understand the relationship between reproductive physiological responses (biochemical and molecular levels) associated with the onset of puberty.

*WP2. Determine the miRNAs biomarkers associated with when the gonad is receptive to recombinant gonadotropins to induce puberty in the farmed flathead grey mullet.*

Recently, several studies have demonstrated the modulation of the miRNA's profiles during the different life stages in several fish species. This innovative approach would lead to finding a post-transcriptional biomarker associated with early maturational development in the farmed flathead grey mullet. For this purpose, blood plasma from the different life stages (juvenile, pubertal and breeders) will be collected. A small RNA-seq next-generation sequencing (NGS) will be run to produce circulating miRNA profiles of blood plasma and to determine the differences among life stages (T2.1). Preparation of small RNA libraries, sequencing on the NextSeq Illumina platform and bioinformatics analyses of the data will be carried during a secondment performed in the Institute of Marine Sciences (ICM-CSIC) in Barcelona (Spain) (T2.2). It is expected to observe putative differences among these groups of animals and to identify possible useful miRNA biomarkers for the onset of puberty and maturation of the farmed flathead grey mullet.

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*WP3. Characterisation of the effect of rGths on the early stages of maturational development in the flathead grey mullet.*
Once a stage of gonadal development that may be receptive to rGths has been defined (physiological, molecular, and epigenetic) in the farmed flathead grey mullet from the previous WPs, a deep study in gametogenesis and quality will be performed in these early breeders. For this purpose, the same group of fish established in the WP1 will be monitored through the early stages of gonadal maturation, and some fish will be sacrificed to perform *in vitro* studies and some will be induced to carry out *in vivo* trials to examine the receptiveness of the gonad to the rGths. For the *in vitro* experiments, individuals will be sacrificed, explants of testis and ovaries will be performed using different rGth concentrations and growth factors to observe the evolution *in vitro*. Periodically, samples will be collected to perform histology, enzymatic activity and molecular analysis. The antioxidant capacity will be determined by the enzymatic activity using protocols adapted for the mullet. Moreover, apoptosis evaluation will be performed by studying the expression by qPCR of anti-apoptotic (*bcl-2*, *bcl-x*, *mcl-1*) and pro-apoptotic genes (*bad*, *bax*, *bak*, *bok*) in the gonads (T3.1). Medium from the cell culture will be collected, and sex steroids will be measured. To observe this evolution *in vivo*, individuals will be initially induced with different doses of rFsh, and early stages of gametogenesis and blood will be extracted to observe the evolution in plasma sex steroids of the animals induced with the different doses (T3.2). It is expected to understand the process of gametogenesis in the farmed flathead grey mullet using this multidisciplinary approach, covering several aspects of the development and germ cell quality.

**Expected results**
This project will provide crucial information about the different signals of the onset of puberty in a promising species for future aquaculture diversification in Europe, flathead grey mullet. Secondly, it will provide the development of gametogenesis at different levels of the BPG axis, which will have a positive impact in mullet and other species production.

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# FROM POND TO PAGE: TEACHING SUSTAINABLE AQUACULTURE THROUGH THE BioAqua COMIC

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## Introduction

Aquaculture is vital for global food security, especially in developing regions where fish is a key protein source. The sector's rapid growth supports millions of livelihoods but is vulnerable to disease outbreaks that threaten aquatic health, ecological safety, and sustainability. Overuse of antibiotics to control diseases raises concerns about antimicrobial resistance and drug residues in the food chain. While ecological aquaculture offers solutions, scaling it without losing productivity is difficult, making innovative, sustainable disease management essential. The COST Action BioAqua tackles this by creating child-friendly educational materials on fish welfare and One Health to inspire future generations toward sustainable aquaculture.

## Material and Methods

The BioAqua comic designed science communication, entitled "Fish Superpowers!", has been crafted to disseminate the state-of-the-art technologies used for sustainable aquaculture to young children at school (non-specialist audiences). It features several chapters explaining the main topics related to aquaculture, food safety, and sustainability. The protagonists of the comic are the three fish heroes in vibrant colours [1], comic style—Finley, Bubbles, and Stella—with George the Farmer and Dr. Clara, the veterinarian. The BioAqua comic introduces complex concepts, *viz*., risk assessment, antibiotic alternatives, and the One Health approach [2] in a straightforward, relatable narrative, through the story of three lively fish and their vet, Dr Clara [3]. As teaching science and literacy together can be challenging, the comic uses fictional characters and stories as tools to spark curiosity and promote equal learning opportunities. This approach follows the principles of Universal Design for Learning and supports lifelong development of pedagogical skills [3]. The colour combinations of green–red, green–brown, blue–purple, green–blue, light green–yellow, blue–grey, green–grey, and green–black were avoided, keeping in mind the individuals with colour-blindness (protanopia, deuteranopia, and tritanopia). High contrast was used in hue, saturation, and brightness; textures or patterns were added to visuals. The comic uses the Open Dyslexic font to make reading easier for children with dyslexia. However, studies suggest it may not always lead to a significant improvement in reading speed or accuracy.[4]. Storytelling choices were driven by the need to balance emotion and interactivity [5]: Finley reflects the readers' curiosity [6], Bubbles expresses caution (mirroring natural scepticism), and Stella represents excitement and optimism. These emotional anchors allow children to connect with the story through different entry points [5]. If a storyteller teaches children, they are encouraged to ask "guess what happens next" at the end of each chapter, enabling prediction and critical thinking [3].

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## Results

The comic provides multiple educational sources: Science education (fun introduction to microbiology, nutrition, and environmental science), Health education (lessons on why food safety and hygiene matter), Civic and ecological education (understanding sustainability, shared responsibility, and the One Health concept). The comic presents six chapters: Chapter 1: The Mystery of the Tired Fins: Our fish heroes start feeling sluggish and confused, prompting a visit from Dr. Clara to find out what is wrong. →This opening chapter introduces the idea that good nutrition is central to keeping fish healthy and thriving. Chapter 2: A Visit from Dr. Clara: Dr. Clara introduces a new "super feed", enriched with safe biomolecular ingredients → Reflects innovation in fish nutrition and science communication. Chapter 3: Testing the Super Feed: The fish eat the feed and experience positive effects: more energy, shinier scales, clearer thinking → Demonstrates measurable benefits of safe, bio-based interventions. Chapter 4: One Health: The fish discover that keeping them healthy also protects the people who eat them and the water they live in → Highlights BioAqua's integrative approach to health. Chapter 5: Aquaculture Matters: George explains aquaculture's role in global food production. The fish feel proud of their role → Encourages understanding of aquaculture as a sustainable food system. Chapter 6: A Cleaner Future: The fish reflect on the importance of sustainable farming practices and clean water → Emphasises eco-friendly aquaculture aligned with EU Green Deal goals.

The link to download the comic is the following: http://hdl.handle.net/10197/28717.

## Conclusions

This educational comic communication serves to promote awareness about aquaculture and essential aspects associated with this critical practice by combining humour, an emotional approach, as well as vibrant visuals. The comic highlights the importance of nutritious, safe feed in maintaining fish health and environmental protection. It encourages young readers to see the connections between personal choices, animal well-being, and global sustainability in a fun and accessible manner. Moreover, the use of relatable characters and storytelling helps demystify complex scientific concepts, making them more approachable and memorable for children. The inclusive design considerations, such as colour-blind friendly palettes and dyslexia-supportive fonts, demonstrate a commitment to accessibility, ensuring the material can reach diverse learners with varying needs.

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# RELEVANCE OF BARLEY PROTEIN CONCENTRATE FOR FEEDING OF AFRICAN CATFISH JUVENILES: GROWTH PERFORMANCE AND BIOCHEMICAL PARAMETERS

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**Introduction**

More than 95% of soymeal, a popular component of animal feed, is imported. Due to restrained soybean production in Europe, as consequence, the fish feed production needs new sources of protein to satisfy their requirements. The protein concentrates have the advantage over whole meals as the non-digestible fibre is eliminated which allow for the possible use of higher levels of plant material in fish diets. Barley protein concentrate (BPC), as a new alternative ingredient in aquaculture, has rarely been tested in fish. By production up to 150 million tons, barley is among the top four produced grains worldwide. The specific composition can vary depending on the processing method and the purity of the concentrate. In the factory of Pannonia Bio Ltd. at Dunaföldvár, Hungary the annual production reached 45 thousand tons. In the frame of AQUAEXCEL TNA project, we aimed to determine the optimum inclusion level of BPC as novel plant protein source, and to assess the best soybean supplementation ratio in the diet of African catfish.

**Materials and methods**

Five isonitrogenous (450 g/kg) and isolipidic (110 g/kg) diets were formulated for African catfish juveniles (*Clarias gariepinus* × *Heterobranchus longifilis*). The control diet (CTR) contained similar ingredients as are used in commercial fish diets, in the other four experimental treatments soybean flour and SPC was replaced by barley protein concentrate in 25-50-75-100%. According to this the BPC content of the diets was 5-10-15-20% (Table 1.). All diets were supplemented with fish premix to ensure the limiting micronutrients and amino acids. The feeding trials involving 1500 juvenile fish (average body weight of 36.5 ± 0.6 g) lasted ten weeks, each replicated three times (90 fish per tank) and reared in a recirculation aquaculture system equipped with 1 $m^3$ fiberglass tanks. During the trials, the fish were fed at a 3% feeding rate using automatic feeders. Water quality parameters (temperature, dissolved oxygen, and pH) were monitored regularly, while nutrients (ammonia, nitrites, and nitrates) were monitored on a weekly basis. At the end of trial samples for digestive enzymes activity, blood chemistry, ALT and AST activity in the liver, and for gene expression related to the growth and metabolism were taken parallel with growth performance and nutrient utilization indicators.

**Results and Discussion**

After the 10-weeks of feeding statistical differences in terms of growth performance and nutrient protein utilization were observed between treatments. The trial with graded BPC levels (replacing 25–100% of soybean meal) showed no growth reduction at 5–10% BPC inclusion, but at higher levels (15–20% BPC) growth performance declined sharply. Weight gain dropped from 212.8g in control to 156.5g and 111.0g at BPC15 and BPC20, respectively and SGR fell from ~2.9%/day to 2.6 and 2.2%/day at the highest inclusion. FCR remained low (~0.69–0.85) in diets up to treatment BPC15, but jumped to 1.44 in the BPC20 group, indicating poor feed utilization at that extreme inclusion. PER showed a similar pattern: it held steady (~2.8–3.2) in control and 5–15% BPC diets but dropped to 1.65 in the BPC20 group. The CONTR and BPC5 groups exhibited similar growth performance throughout the period, while treatments BPC20 and BPC15 showed a noticeable decline after 28 days of feeding, which became more pronounced at the 49-day sampling time compared to CONTR and BPC5.

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Generally moderate inclusion of alternative proteins in fish diets tends not to drastically disturb basic blood parameters, provided nutritional requirements are met. There were fluctuations in several parameters, such as glucose and total protein but these did not indicate any pathological condition. Various other blood parameters (e.g., urea, creatinine, minerals) generally stay within normal ranges on alternative diets unless the fish are experiencing toxicity or severe metabolic disturbance. The ratio of blood urea nitrogen to creatinine was significantly altered at 20% BPC (8.0 vs ~2.4–4.6 in other groups) which might indicate a subtle shift in protein metabolism at that extreme diet (higher relative blood urea nitrogen suggests more protein catabolism or reduced growth using that protein. Similarly, plasma total cholesterol was affected by diet tended to rise with 10–20% BPC inclusion (132.6 mg/dL at BPC20 vs 116 mg/dL in CONTR) comparable with study on African catfish fed with mealworm containing diet (Gebremichael et al, 2023). Despite these changes, cholesterol values remained in a moderate range, and other liver function indicators (like bilirubin or bile acids) were normal, suggesting no severe liver impairment. In Atlantic salmon, inclusion of barley protein caused no reported abnormalities in blood metabolites – consistent with the lack of antinutritional factors in BPC (Bell et al, 2016). In conclusion, based on growth and nutrient utilization parameters, as well as blood biochemical analyses, it can be said that feeding BPC to tested young animals is recommended at a maximum of 10% content.

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# EFFECT OF HIGH SEA WATER REARING TEMPERATURE ON THE ONSET OF EARLY PUBERTY IN MALE AND FEMALE EUROPEAN SEA BASS

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## Introduction

Climate change is a serious concern for the aquatic environment, as it alters the physical and chemical properties of seawater causing negative impacts on fish. Among its many effects, the increase of atmospheric greenhouse gases has led to a rise in average sea surface temperatures. Fish are poikilothermic and are therefore extremely sensitive to changes in environmental temperature. Exposure of adult fish to warm temperatures is known to impair reproduction, although the long-term reproductive impact during early life stages remain poorly understood. The European sea bass (*Dicentrarchus labrax* L.) is a marine species of high commercial value, in which a significant proportion of individuals exhibit precocious puberty during the grow-out phase, when they are reared to marketable size. A situation that aquaculture producers want to prevent. The goal of this study was to evaluate the effect of high sea water rearing temperature on the onset of early puberty in this species. Growth and gonad maturation in juvenile 1-year-old male and 2-year-old female European sea bass were analysed, and its consequences in productive terms were considered.

## Materials and methods

Juvenile sea bass (initial body weight (BW) of 14.0 ± 4.1 g) were provided by Piscialba-Piscifactorías Albadalejo S.L. (Murcia, Spain). Eight-month-old fish were sized and weighed, and the smallest 85% of the animals were considered potentially male fish (n = 510 fish) whereas the largest 15% were considered potentially female fish (n = 90). Potential male fish were distributed in six 500-l circular fiberglass tanks (n=85 fish/tank) whereas potential female fish were distributed in two 500-l circular fiberglass tanks (n = 45 fish/tank). After an acclimatization period of 30 days, two experimental groups for each sex were organized in triplicate in males and duplicate in females: control group (CG) that was maintained under natural seawater temperature conditions at the IATS facilities (40°08′15″ N; 0°10′12″ E) and high-temperature group (HTG) that was maintained at a temperature that was 3-4 ºC above that of the CG. The survival rate as well as BW and fork length (FL) of fish was periodically measured. Blood samples were collected, and the plasma was stored at -20ºC until hormonal analysis. In the case of males, in order to assess the testicular growth and the proportion of early maturing fish, 4-10 animals per group were sacrificed from July to February during their first year of life. The ovarian growth and the incidence of early onset puberty was assessed in 4-10 females per group that were sacrificed from October to February during their second year of life. Gonad was removed and weighed to determine the gonadosomatic index (GSI) and small pieces of tissue were fixed for histological analysis and assessment of the stages of testicular and ovarian development. The percentage of early maturing males was also evaluated from November to onwards by applying gentle hand pressure to the abdomen of all fish. Circulating plasma levels of follicle stimulating-hormone (Fsh) and its content in the pituitary were evaluated in both sexes. Plasma levels of testosterone (T), 11-ketotestosterone (11-KT) and estradiol ($E_2$) were also analysed in males, whereas levels of $E_2$ and vitellogenin (Vtg) were analysed in females.

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## Results

After five months of experiment, male sea bass reared at a high temperature (HTG) showed a lower survival rate than that of the CG (66% and 96%, respectively). Males in HTG was approximately 12.61 % lower in weight and 3.14 % smaller in length than that of CG at 17 months of age. The condition factor varied between 1.01 and 1.81 in fish at HTG, while it was around 1.02-2.14 in the CG. First significant increase of the GSI was observed after 4 months of experiment in fish of the CG (GSI= 0.47 ± 0.42%) compared to males reared in HTG (GSI= 0.05 ± 0.03%). In February, during the tentative testicular growth period, GSI of the HTG was 1.66 ± 0.33% whereas values in the CG were significantly higher (2.74 ± 0.26%). The histological analysis of testis revealed clear differences between CG and HTG, where the percentages of gonadal stage III (mid recrudescence) in the CG reached 17% in October and 57% in November whereas the HTG group displayed 14.3% in December. In February, the percentages of gonadal stage IV (late recrudescence) in the CG was 89% and 11% in stage V (full spermiating testis) where all males in the HTG reached gonadal stage IV. Furthermore, the proportion of precociously spermiating males was higher in the CG than that of HTG from December to March, with a peak of 78% in the CG in comparison to 23% in the HTG in February. Of note, spermiation time lasted one month longer, until April, in the CG. Also, males in the HTG exhibited an increase of plasma and pituitary content of Fsh, whereas the levels of T and 11-KT remained unchanged in both experimental groups. In the case of females, no significant differences in growth performance were observed between CG and HTG. The condition factor ranged from 1.19 and 1.77 in the HTG and from 1.18-1.61 in the CG group. Although values of GSI were higher in the CG compared to those of HTG, no significant differences were observed. In February, values of GSI of the HTG were 3.65 ± 1.04% whereas those of the CG reached 6.58 ± 0.84%. Histological analysis of ovaries revealed that the incidence of early onset puberty in females was similar in both groups. Accordingly, all females in the CG and HTG showed oocytes in late vitellogenesis in February, although the animals in the HTG group entered vitellogenesis one month later than those in the GC group. Finally, females in both experimental groups exhibited similar Fsh and $E_2$ plasma levels, whereas the levels of vitellogenin increase later in the HTG group, aligning with the delayed vitellogenesis observed histologically.

## Discussion

These findings show that high sea water rearing temperatures reduce the long-term growth performance of juvenile male European sea bass during their first year of life. Although males can start spermatogenesis, fish in the HTG show a delay in the progression towards advanced testicular stages and a reduction in the time fish remain in spermiation. On the other hand, high sea water rearing temperatures do not affect growth performance of female sea bass during their first two years of life and the incidence of early puberty is similar in both CG and HTG groups. Accordingly, high sea water rearing temperatures affect the reproductive axis of European sea bass at multiple levels, although the occurrence of early sexual maturation persists and, in turn, its side effects need to be avoided in terms of production. Finally, high sea water rearing temperatures can impact spawning season, sometimes shortening it, thus influencing the life history traits of fish species and populations.

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# MUSCLE FATTY ACID COMPOSITION OF IMMATURE AND MATURING EUROPEAN SEA BASS

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## Introduction

The European sea bass (*Dicentrarchus labrax* L.) is one of the most important marine fish species consumed in Europe, primarily due to its affordable market price, appealing aroma, and desirable flesh quality. Several studies have highlighted differences in the chemical composition between wild and farmed sea bass. Moreover, deterioration in fillet texture during sexual maturation is a well-documented concern in aquaculture species, posing a challenge to the industry when marketing fish for consumption. It has also been suggested that certain flesh quality traits in farmed fish may be influenced by lifestyle or the sheltered conditions characteristic of aquaculture systems. In the specific context of sexual maturation, the accepted, but scarcely proven paradigm is that this biological process reduces meat quality by altering fatty acid composition—particularly due to the mobilization of polyunsaturated fatty acids (PUFAs) to meet the gonadal requirements. Early sexual maturation in European sea bass is known to negatively impact growth, resulting in size variation and reduced overall biomass. However, there is a lack of data regarding the direct effects of sexual maturation on muscle lipid content, and especially on fatty acid composition. Accordingly, the aim of this study was to evaluate the effects of early sexual maturation in immature and maturing male and female sea bass, using the muscle fatty acid profile as an indicator of fillet quality in this species.

## Materials and methods

Fish were sampled in February, coinciding with the reproductive season of *Dicentrarchus labrax*. A total of 57 individual muscle samples (4 cm × 2 cm) were collected from the anterior left dorsal muscle and stored at −80 °C until analysis. Total lipids were extracted from freeze-dried tissue using the method of Folch et al. (1957), then transmethylated and analyzed by gas chromatography (Guinot et al., 2013). The sexual status of each fish was determined histologically, based on the developmental stage of the testes or ovaries. Specifically, 17 immature and 12 maturing one-year-old males were classified, along with 12 maturing two-year-old males. Among females, 8 immature and 3 maturing two-year-old fish were identified, as well as 5 maturing three-year-old individuals. Fatty acid profiles were analyzed using Principal Component Analysis (PCA), which was applied to highlight segregation among the different experimental groups—specifically according to sex, age, and degree of sexual maturity.

## Results

Muscle lipid content and fatty acid composition in immature and maturing (precocious) one-year-old male European sea bass showed no significant changes. However, regardless of reproductive stage, significant differences in fatty acid composition were observed between one- and two-year-old males. Similarly, no significant changes were detected in the muscle lipid content or fatty acid composition of immature and maturing two-year-old females. In contrast, two-year-old females exhibited marked differences in fatty acid composition when compared to three-year-old females. Overall, the findings of this study did not indicate a drastic change in the flesh fatty acid profiles associated with sexual maturation, as the observed differences were not linked to reproductive status or sex. Instead, the results suggest a cumulative dietary effect on the muscle fatty acid profile, contributing to the age-related differences in fatty acid composition observed among the fish.

## Discussion

This study provides novel insights into the fatty acid composition associated with sexual maturation in European sea bass. The findings show no significant differences in muscle lipid content or fatty acid composition between immature and maturing individuals of the same age, nor between males and females. While age-related differences in fatty acid profiles were observed, these were not linked to reproductive status. This suggests that early sexual maturation does not markedly affect fillet quality in this species, and thus, the associated economic impact on aquaculture may be less substantial than previously assumed.

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# SIZE-SPECIFIC GUT MUCOSAL RESPONSES TO ALGAE-DERIVED FEED ADDITIVES IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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**Introduction**

The sustainable intensification of aquaculture production is fundamentally dependent on the capacity of cultivated fish to adeptly manage a range of environmental and physiological stressors and challenges. Preserving optimal health and resilience in aquatic organisms is of utmost importance for productivity, welfare, and the sustainability of the sector. In this regard, the development of functional feeds constitutes a promising and ecologically sound strategy aimed at enhancing fish health and fortifying their innate defense mechanisms. Algae are gaining prominence in this field, as they provide an abundant source of bioactive compounds with considerable potential as functional ingredients. Considering the pivotal role of the gut mucosa in fish health and homeostasis, a positive interaction of algae-derived ingredients with the fish's intestinal mucosa will support a modulation of its functions towards performance and fish health improvement. Here we used a European seabass *ex vivo* model derived from either young juveniles or adults, to evaluate and categorize the functional properties of *Ulva* sp. broken biomass (*Ulva*-BC), together with two commercial products based either on seaweed-derived sulfated polysaccharides or algae-derived beta-glucans. Special focus was given to the immunostimulant and antioxidant capacity, but also on the intestinal mucosa integrity and homeostasis promotion testing for a differential response according to fish size.

**Material and methods**

To infer if fish respond similarly in different stages, we used an optimized *ex vivo* gut mucosal screening model based on intestinal explants, to assess the transcriptional responses of small (27 - 44 *g*) and large (152 - 180 *g*) European seabass to the ingredients. Fish were fasted for 24 hours and anesthetized for explants excision from the anterior intestine. These were then incubated with a commercial-like dosage of each target ingredient for 4 hours. To determine size-specific effects, tissue response was evaluated by quantitative real-time PCR (qPCR) of genes associated with immune response (*il1b*, *il8*, *tnfa*, *mmp9*, *mmp13*), antioxidant defense (*sod1*, *gpx1*, *ptx*) and epithelial integrity (*tjp1*, *ocl*, *cldn5*, *cldn12, imuc*). Gene relative expression was calculated, and levels were then compared between small and large fishes.

**Results**

We observed that the gut mucosa of small seabass exhibited a different expression profile of immune-related genes compared to larger individuals. In both size groups, the commercial products did not significantly upregulate immune gene expression. Instead, only slight activation or maintenance of immune markers was observed, suggesting a potential modulatory rather than a strong immunostimulatory effect. On the other hand, *Ulva*-BC induces a robust immune response, mainly in larger fish (with higher expression of *il1b*, *il8*, *mmp9*), and to a lesser extent in smaller fish (mainly through *il1b* and *mmp9*). This highlights its potential for enhancing immunocompetence during grow-out phases. No differences were observed in antioxidant responses between small and large fish. Interestingly, molecular markers associated with epithelium integrity and homeostasis were either not affected or, in some cases, downregulated following the inclusion of *Ulva*-BC and the product with seaweed-derived sulfated polysaccharides. On the other hand, the algae-derived B-glucan enriched ingredient stimulated the expression of mucosa integrity markers (*ocl*, *tjp1*, *imuc*, *cldn5*), although at a lower magnitude. This indicates that although all ingredients were algae-derived, their effects differ in both application and intensity depending on fish life stage

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**Conclusion**

Based on these findings, algae-derived ingredients have a promising, yet stage-specific, role in enhancing European seabass health. While the commercial products showed only a mild immunostimulatory effect, the *Ulva*-BC showed strong potential to boost immunity, especially in larger, more mature fish. This makes it particularly useful during the grow-out phase, when fish face more environmental stressors. The different responses to the ingredients highlight a critical distinction: not all algae-based products work the same way. The algae-derived beta-glucan ingredient was especially effective at improving epithelial integrity, while the other ingredients showed little to no effect. These results emphasize the importance of a targeted approach to functional feed development, that align specific bioactive compounds to the fish's developmental stage to improve health and promote sustainable aquaculture.

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# TRPV CHANNELS IN TESTES AND SPERM: DESCRIBING THE PHYSIOLOGICAL EFFECTS OF RAISING WATER TEMPERATURES IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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## Introduction

The transient receptor potential cation channels (TRPV) subfamily of ion channels is crucial in mediating temperature-sensitive physiological processes in fish species. In teleost, *in silico* analyses have identified three to four members of the TRPV subfamily: TRPV1 (TRPV1a and TRPV1b in some species), TRPV4 and TRPV5 (Morini et al., 2022). As global climate change drives elevated water temperatures, concerns have grown over the potential impacts on gonadal development and spermatogenesis. To explore this, we studied how TRPV expression relates to androgens and sperm kinetics in male sea bass during their second year of growth, after being raised in warm water from an early age.

## Material and methods

Characterization of TRPV in European sea bass was made *in silico* by using BLAST tool from NCBI. Gene-specific primers for TRPV and the reference genes —elongation factor 1-alpha (ef1-α) and ribosomal protein L13 (rpL13) — were designed for quantitative PCR. Juvenile sea bass (average initial weight: 14.0±4.1 g) were divided into two treatment groups, each in triplicate for 21 months: (i) a control group (CG) reared at ambient seawater temperature (40°08′15″ N; 0°10′12″ E), and (ii) a high temperature group (HTG), maintained at 3–4 °C above the control temperature. To assess the reproductive influence of temperature a total of 10 fish (5 males each group) were sampled at three key stages of gonadal development: early recrudescence (October), late recrudescence (December), and final maturation-spermiating testes (February and March). Circulating blood levels of testosterone (T) and 11-ketotestosterone (11-KT) were measured by a conventional enzyme immunoassay (EIA), and TRPV expression from testes were measured monthly by quantitative PCR, while sperm kinetics (MOT, MOTp VAP, VCL, and VSL) were evaluated using Computer-Assisted Sperm Analysis software during the spermiating season, in March.

## Results and discussion

Our investigation showed for the first time the presence and expression of four paralogous genes belonging to the TRPV subfamily in European seabass: TRPV1a, TRPV1b, TRPV4, and TRPV5.

In October, TRPV1b and TRPV4 showed significantly higher expression levels in the HTG (average monthly temperature: 25.8 ºC) compared to the CG (21.7 ºC), suggesting the existence of a temperature threshold for these genes in sea bass. Additionally, different expression patterns were noted within the HTG: TRPV1a and TRPV1b showed its maximum expression in October, whereas TRPV4 and TRPV5 exhibited a cyclical expression pattern, reaching its maximum expression at the onset and the end of the reproductive season. A principal component analysis (PCA) revealed a distinct clustering of TRPV and circulating steroids levels under high rearing temperature, suggesting that male reproduction is a temperature-sensitive process involving these receptors. By the end of the reproductive period, correlations between gene expression, circulating steroids levels and sperm kinetics highlighted the involvement of TRPV in reproductive performance: the paralogs TRPV1a and TRPV1b displayed an antagonistic role in sperm function, while TRPV1a was associated with boosted sperm performance, high expression levels of TRPV1b were clearly correlated with the reduction of sperm kinetics (Fig. 1A, B). Given its correlation with T and 11-KT (Fig. 1B), we suggest that TRPV1b might be involved during sexual maturation and early stages of spermatogenesis whereas TRPV1a seems to be critical during spermiation. Although a positive correlation was observed between TRPV1a, TRPV4 and VAP in CG (Fig. 1A), the increased temperature modified this interaction, leading TRPV4 to align with TRPV1b, resulting in a negative impact on spermatozoa velocity (Fig. 1B).

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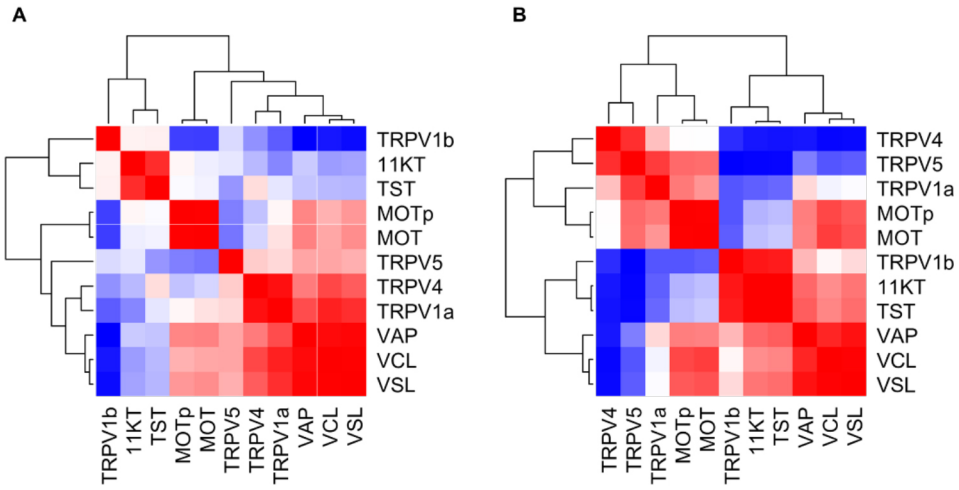


Figure 1. Clustered heatmap based on Pearson correlation analysis ($p < 0.05$) of sperm quality, blood steroids level and gene expression in gonad of full spermiating sea bass males from CG (A) and HTG (B). Red indicates a positive correlation while blue indicates a negative correlation. The darker the colour, the greater the correlation coefficient.

Overall, our findings suggest the involvement of TRPV in the reproductive physiology of male sea bass and demonstrate that elevated rearing temperatures can alter their function. Nevertheless, additional studies are required to clarify the specific molecular pathways involved in TRPV-mediated responses and to better understand their impact on reproductive outcomes in a global warming scenario.

**Acknowledgments**

This study forms part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (GVA-THINKINAZUL/2021/012; Principal investigator: J.F. Asturiano, Universitat Politècnica de València, and GVA-THINKINAZUL/2021/042; Principal investigator: A. Gómez, CSIC). MM is a recipient of a Seal of Excellence MSCA Post-doctoral Fellowship from Sorbonne University. S.S. was supported by a research contract from Margarita Salas program from Universidad de Las Palmas de Gran Canaria.

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# MACROALGAE-BASED BIOREMEDIATION AND BLOCKCHAIN CARBON CREDITS: A PILOT NATURE-BASED SOLUTION FOR PORT WATERS

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## Introduction

The removal of heavy metals and nutrients from port waters remains a critical environmental challenge. In this line, macroalgae bioremediation has demonstrated potential to enhance water quality and support biodiversity. Moreover, when integrated with ICT tools, such as carbon footprint tokenization, blockchain and IoT monitoring, it also generates economic incentives for sustainable practices. However, broader implementation still demands evaluation of regulatory, economic and operational feasibility.

## Material and Methods

This pilot study, conducted at the Port of Alicante in collaboration with Mediterranean Algae company, tests the use of *Ulva sp*. cultivation combined with ICT tools for port water treatment. The system uses circulating tanks to grow macroalgae while monitoring environmental parameters. It aims to enhance water quality and address climate change impacts by providing Blue Carbon Credits.

The pilot consists of three 2000L tanks where *Ulva sp*. is cultivated to treat port water through continuous circulation and bioremediation in a closed system. IoT sensors monitor key parameters, while a digital platform enables real-time system optimization. $CO_2$ absorption is tracked via blockchain to generate carbon credits, creating economic incentives. *Ulva sp*. is analyzed for contaminants and explored for reuse, linking bioremediation with waste valorization and scalability.

## Discussion

The pilot will integrate macroalgae and digital technologies to improve water bioremediation and promote circular economy models through Blue Carbon Credits generation. It will identify native algae potential, develop new algae-based products and enhance public-private collaboration. A replicable protocol will be developed to support future implementation and scalability in other regions.

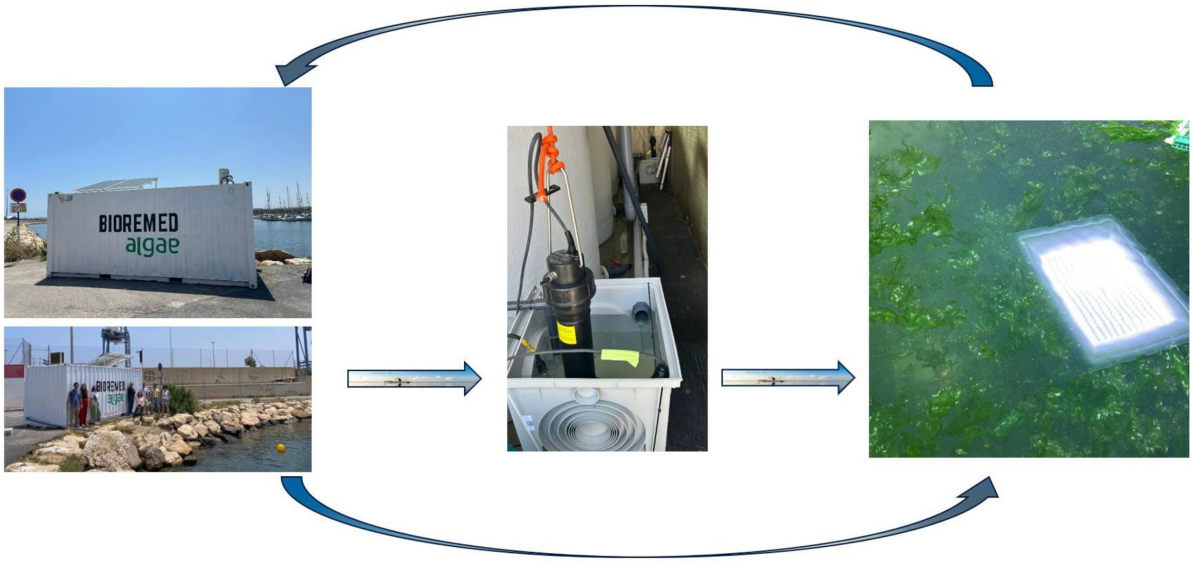


*Figure 1. Material and Methods*

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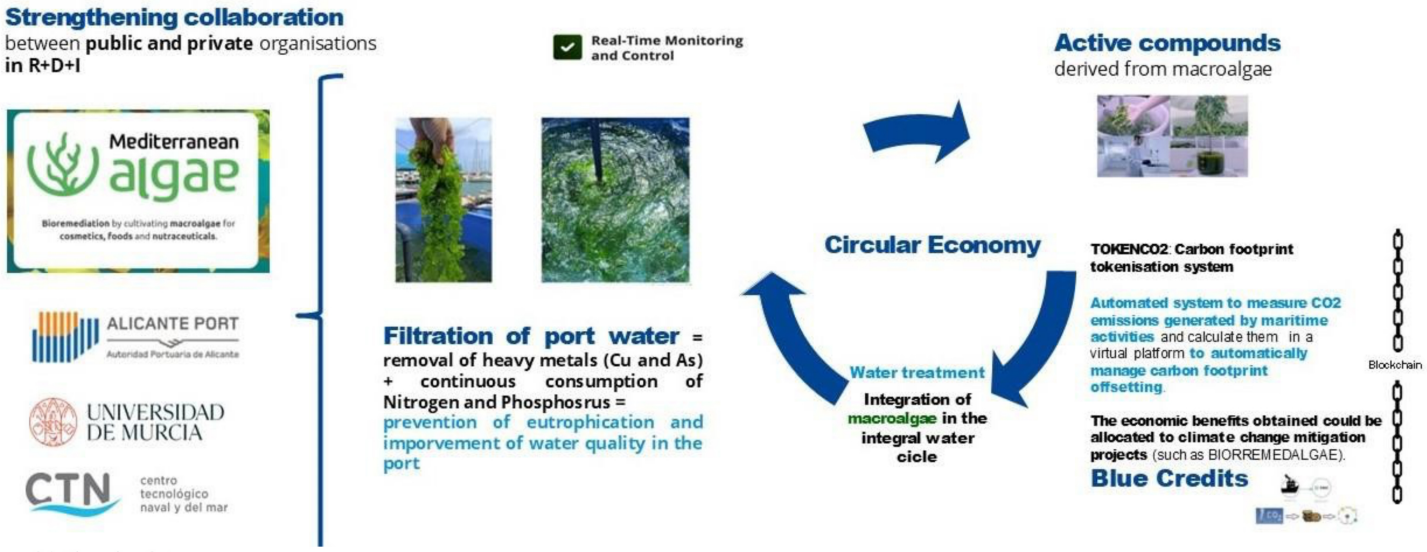


*Figure 2 1: Discussion. Outcomes.*

| Perfomance Indicators |
|---|
| Reduction in Nutrient Emissions |
| Water Quality Enhancement |
| $CO_2$ Sequestration Efficiency |
| Biodiversity Enhancement |
| Nutrient Removal Efficiency |
| Biomass Production Output |
| Number of new products marketable |

*Table 1 Discussion. Expected outcomes*

**Acknowledgements**

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# EFFECT OF CARVACROL FEED SUPPLEMENTATION ON MELANOSIS OF WHITE SHRIMP (*Litopenaeaus vannamei*)

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## Introduction

White shrimp (*Litopenaeaus vannamei*) is a very important aquaculture species worldwide. These crustaceans have a very short shelf-life, mainly due to microbial spoilage and also to melanosis, the latter being a major problem for shrimp marketing. To prevent melanosis, sulphites are commonly used. However, they are associated with allergic reactions, and consumers are increasingly seeking additive-free foods. There is a growing interest in natural alternatives that prevent melanosis and can extend shrimp shelf-life. Essential oils or their compounds have been studied in different forms of application, such as in active films or by spraying or immersing shrimps in their solutions, demonstrating the great potential of these compounds to delay melanosis. The objective of this work was to study the effect of the carvacrol (free or encapsulated in montmorillonite) supplemented in shrimp feed, on the prevention of melanosis.

## Materials and methods

Three different shrimp feed formulations were studied: A control diet (**Control**, 33% crude protein and 10% crude lipid), the same diet supplemented with 150 mg/kg of carvacrol in free form (**Car_Free**), and the last one supplemented with 150 mg/kg of carvacrol encapsulated in montmorillonite (**Car_MM**).

Before starting the feeding test, all shrimp larvae were acclimated to indoor rearing conditions for four weeks and fed a standard diet. The trial began with shrimp weighing approximately 1.8 g. Three aquariums were used per diet, each containing 25 specimens. The shrimp were hand-fed three times a day for a total of 78 days. After this period, the shrimp were slaughtered and stored in refrigeration for 10 days. The melanosis was evaluated on days 0, 3, 6, and 10 of storage., by visual inspection by 10 trained panellists. The scale of melanosis used was: Absent=0; Mild=up to 20 %; Moderate=20-40 %; Notable=40-60 %; Severe=60–80 %; Extensive=80–100 %.

## Results

Melanosis scores are shown in Fig 1. The highest scores were obtained in shrimps fed the control diets for all storage days, except for day 0, where no melanosis was observed. The main differences were observed on days 3 and 6, with the sample fed the diet with encapsulated carvacrol (Car_MM) presenting the lowest scores, although there were no significant differences between Car_Free and Car_MM. At day 10 the differences between samples were smaller, with no significant differences between them.

## Conclusion

Carvacrol encapsulated in montmorillonite shows potential as a feed ingredient to prevent melanosis; however, at the concentration used in this work, the effect is not enough to prolong shrimp shelf-life. Further studies with higher concentrations should be carried out to determine if this compound could prevent the problem of melanosis.

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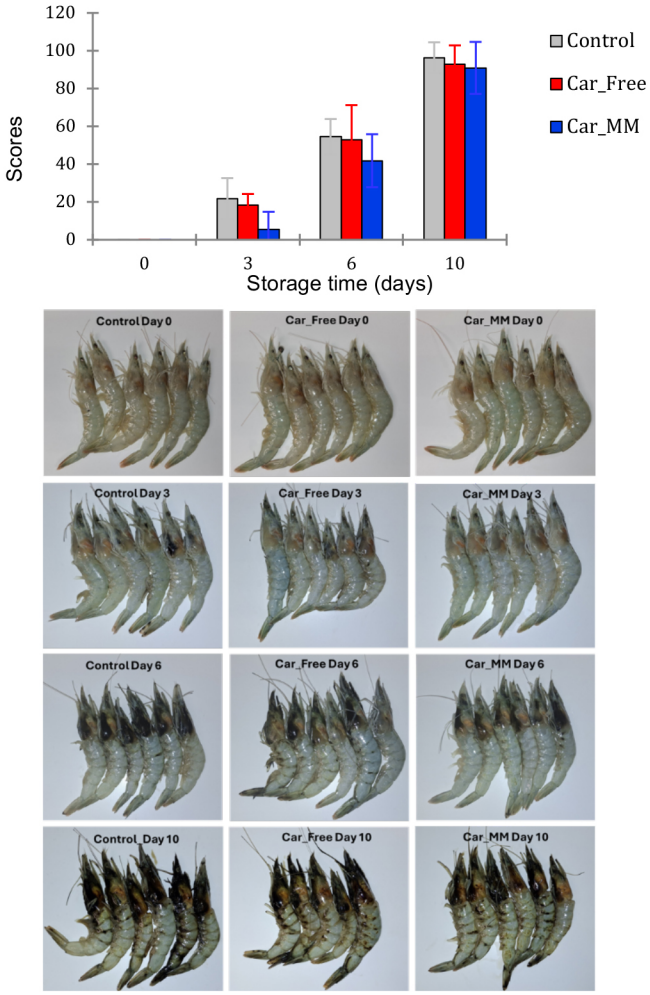


Fig. 1. Melanosis scores and photographs of white shrimps fed the different diets (Control, supplemented with free carvacrol or with carvacrol encapsulated in montmorillonite (MM)) during 10 days of storage.

**Acknowledgements**

This study forms part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (GVA-THINKINAZUL/2021/006; M. Jover; D. Sánchez, GVA-THINKINAZUL/2021/004; J.M. Barat; I. Fernández-Segovia, UPV).

# EVOLUTION OF MICROBIAL QUALITY OF FISH NUGGETS CONTAINING FISH BY-PRODUCTS

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## Introduction

The fishing sector, including aquaculture, is one of the food production sectors that has experienced the greatest growth in recent years. The high production volume leads to the generation of a large amount of by-products. In the case of sea bream (*Sparus aurata*), the filleting process generates by-products including skin, bones, and heads, which are often not properly utilized and are discarded (Rathod et al., 2024). Fish is an essential food in the diet, so it is important to promote its consumption, particularly among population groups with low fishery product intake, such as children. Fish nuggets represent an attractive alternative due to their ease of consumption and sensory acceptability. In this context, sea bream nuggets made with fishery by-product meal emerge as an innovative option, utilizing fishing industry waste to improve sustainability and nutritional value. Consumers' search for fresh products makes the marketing of nuggets on refrigerated shelves attractive. However, the commercial potential of these products is affected by their shelf life, which is limited primarily by microbial growth.

The objective of this study was to study the microbial and sensory quality evolution of sea bream nuggets which contained fishery by-products, packaged in a modified atmosphere in cold storage.

## Materials and methods

Cleaned and gutted sea breams were filleted, using the muscle to make the nuggets and the by-products to obtain the flour. Fish by-products were boiled in water, drained, pressed with cheesecloth, grounded, dried at 60 °C, milled and sieved. Different nuggets formulations were developed: C (control samples with sea bream muscle, salt, potato flakes, egg white and ice), B (samples C where part of the seabream flesh was replaced by 6 % of fish by-products meal). The batter ingredients were: salt, xanthan gum, baking powder, baking soda and water. Breadcrumbs were used to coat the nuggets.

Samples were packaged in trays in modified atmosphere (MAP) (70% $CO_2$ and 30% $N_2$) and stored under refrigeration (4 °C) during 21 days. Microbiological analyses (aerobic mesophilic bacteria and *Enterobacteriaceae*) were performed on days 0, 3, 7, 10, 14, 17, and 21 of storage.

## Results

The mesophilic and *Enterobacteriaceae* counts in the two types of sea bream nuggets are shown in Fig. 1. The initial level of mesophilic bacteria was below 4 log CFU/g for both formulations, indicating a good hygienic-sanitary quality of the freshly prepared samples. Counts of these microorganisms increased progressively during storage. From day 14 onwards, both samples exceeded the acceptable limit, showing counts greater than 7 log CFU/g.

Regarding the evolution of *Enterobacteriaceae* growth, a significant increase was observed during storage. In this case, the trend is similar to that for the growth of mesophilic microorganisms, exceeding the acceptable limit from day 14 onwards, with counts greater than 3 log CFU/g.

The incorporation of by-product meal in the formulation did not imply significant differences in either the mesophilic or *Enterobacteriaceae* counts.

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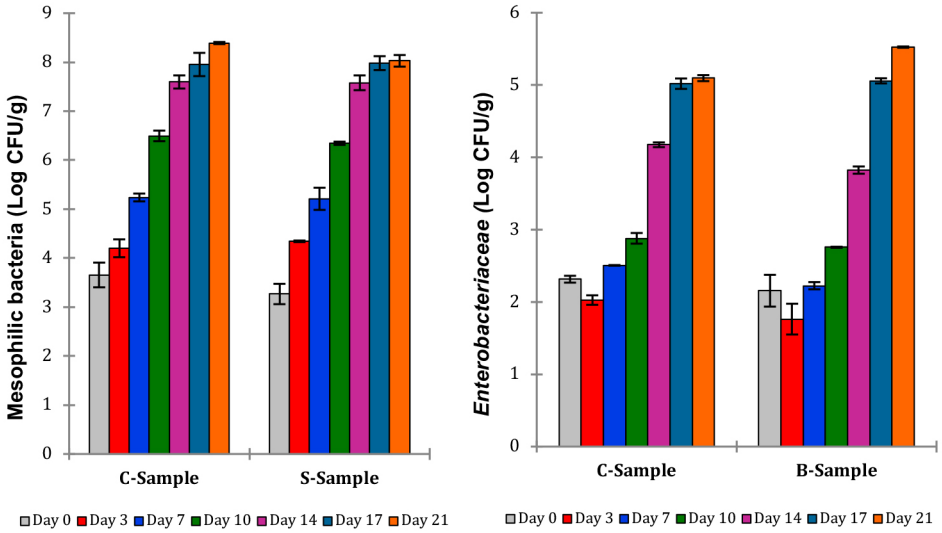


Fig. 1. Mesophilic and *Enterobacteriaceae* counts in the two types of sea bream nuggets (control (C) and by-product (B)) during the 21 days of storage. Error bars represent standard deviations.

## Conclusions

Sea bream nuggets with or without by-products packaged in MAP in cold storage would have a shelf life of 10 days. According to these results, incorporation of by-product meal in nuggets formulations does not affect the products' shelf life.

## Acknowledgements

This study forms part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (GVA-THINKINAZUL/2021/004; J.M. Barat; Fernández-Segovia, I., UPV).

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# ADVANCES IN THE CULTIVATION OF STRIPED RED MULLET *Mullus surmuletus*: COLOR CHANGE INDUCTION

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Introduction

The main objective of the SALROC research project is to contribute to the diversification of marine fish farming in Europe by optimizing farming techniques for the striped red mullet (*Mullus surmuletus* Linnaeus, 1758). This project, funded by the Xunta de Galicia, has been running since 2024 at CIMA (Centro de Investigacións Mariñas de Galicia) and CETGA (Centro Tecnológico del Clúster de la Acuicultura).

During the initial investigations of the SALROC project, it was found that striped red mullets housed in CETGA adopted differents color shades depending on the color of the inside of the tanks (black or green) where they were housed. In CIMA tanks, with white interior walls, the specimens progressively lost their pinkish-reddish coloration, taking on whitish tones. To study the effects of tank color on the body color of the striped red mullet, raffia nets of differents colors was placed on the bottom of the CIMA tanks.

**Material and methods**

This experiment was carried out in three cylindrical tanks, each 1.10 m in diameter, with a surface area of 1 $m^2$ and a volume of 0.5 $m^3$, located at the CIMA. Twelve individuals, captured in the Ría de Arousa (NW Spain), were housed in each tank. The tanks were originally designed for mollusc culture but were adapted to accommodate flatfish and benthic fish (Fernández and Brianes, 2025). Bottom refuges made from oyster baskets were installed at the bottom of the tank as Environmental Enrichment (EE) elements (Fernández *et al*., 2025).

The interior walls and the background of the CIMA tanks were white, which allowed for various color change tests. In February 2025, to study the effects of tank color on the body color of striped red mullets, to verify the intensity and speed at which these color changes occurred, and to determine their possible reversibility, plastic raffia nets of different colors (green, brown and black) were temporarily placed on the white bottom of the tanks at CIMA.

**Results**

Following the placement of raffia at the base of the CIMA tanks, a rapid and significant alteration in the fish's pigmentation was observed. The intensity of the colouration increased proportionally with the darkness of the raffia substrate, with individuals exhibiting vivid reddish hues when black raffia was utilised, and comparatively less intense reddish tones when green or brown raffia was employed (Figure 1). Additionally, the sclera of the striped red mullet's eyes exhibited a reddish coloration (Figure 2). These chromatic changes occurred swiftly, within less than five minutes, and were reversible; upon removal of the coloured raffia, the specimens reverted to a pale body pigmentation and the sclera returned to its white appearance within minutes., showing an amazing camouflage power ("*the chameleon of the sea*").

Being able to easily induce the camouflage ability of striped red mullets with their surroundings so that they quickly adopt a more "natural" color is of great importance for their potential cultivation, since an appropriate color of the fish (close to that typical of wild specimens) is one of the characteristics that could facilitate the future commercialization of this species.

**Conclusions**

The integumentary coloration of fish (and other animals) is primarily driven by the presence of various types of chromatophores, which in some cases allow them to adapt chromatically to environmental tones and patterns through mimicry or camouflage (Fujii, 2000). Studies on changes in fish color patterns are relatively abundant, especially in flatfish, but not in the case of the striped red mullet (Tokac *et al*., 2013).

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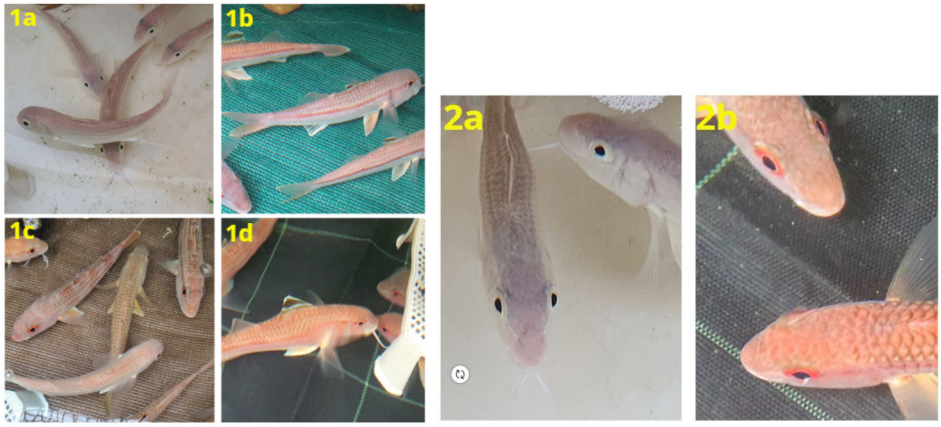


**Figure 1**: Striped red mullets on white (1a), green (1b), brown (1c) and black (1d) backgrounds. **Figure 2**: Detail of the change in color of the sclera of the eye of the striped red mullets in a tank with a white background (2a) and after placing a black raffia on the bottom of the culture tank (2b).

This study demonstrates for the first time a phenomenon of pigmentation change in striped red mullet housed in culture tanks, opening up the possibility of applying camouflage-inducing techniques by changing the color of the bottom and/or internal walls of the culture tank, either temporarily (by placing raffia on the bottom) or permanently (by painting the interior of the tank). Color changes toward more consumer-friendly hues could be induced by using dark tanks throughout the entire culture cycle of the species or by temporarily housing them (a few days before marketing) in dark-colored tanks. This experience also opens the door to future research that could determine the optimal color for culture tanks for this species from an animal welfare perspective.

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# GROWTH OF *Ruditapes philippinarum* IN LAND-BASED PONDS AS AN ALTERNATIVE TO AQUACULTURE AT SEA DURING THE *Callinectes sapidus* INVASION

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## Introduction

The blue crab (*Callinectes sapidus*) invasion in the Western Mediterranean Sea has strongly impacted Manila clam (*Ruditapes philippinarum*) aquaculture (Chiesa et al., 2025; Prado et al., 2024). Manila clam aquaculture in land-based ponds is an alternative that allows us to avoid the risk of predation from the blue crab. Pond integrated multi-trophic aquaculture (IMTA) appears as a further step to increase productivity through the efficient recycling and reuse of nutrients. Manila clam growth in IMTA earthen experimental ponds in the Ebro delta has shown to result in improved growth performance compared to Manila clam pond monoculture. Higher particulate organic matter content in the water due to the fish fed supplied and microphytobenthos resuspension by the fish in the pond may explain the big differences in clams growth performance.

## Materials and methods

A Manila clam monoculture experiment was carried out in an earthen pond (20 m x 5 m) covered with a thin layer of sand filled with seawater up to 0.30 m; seawater from Alfacs Bay (Ebro delta, Western Mediterranean) nearshore waters was pumped at a rate of one renewal per day (30,000 L/day). The clams were seeded (16.5 ± 1 mm) in March 2020 at a density of 50 clams per $m^2$, harvesting was conducted in November 2021. The pond was covered with a sunshade netting, during summer months, to reduce the growth of macroalgae and decrease seawater temperature. Physicochemical parameters were measured twice per day, morning and afternoon, chlorophyll-*a* concentration was analyzed daily, clams growth was measured once per month during the whole period.

A second experiment was carried out at the same earthen pond, and a duplicate pond next to it, where the Manila clam culture was part of an integrated multi-trophic system that also included flathead grey mullet (*Mugil cephalus*) and oysters (*Ostrea edulis* and *Crassostrea gigas*). Seawater from Alfacs bay was pumped at a rate of 96,000 L/day. The water depth was 1 m. In this second experiment the ponds were not covered with sunshade nets. Mullet was fed with fish feed. Clam seeding (18.7 mm ± 1 mm) was conducted in November 2024 at a density of 30 clams per $m^2$, 1,000 individuals of *O. edulis* and 1,000 individuals of *C. gigas* per pond were seeded at the same time. Physicochemical parameters were measured daily, chlorophyll-*a* concentration was analyzed twice per week, clams growth was measured in March, May, June and August 2025.

To sample each pond, we collected the clams present in 10 replicates using 25 × 25 cm quadrats. Clams per quadrat were counted, the shell length of the clams was measured with a caliper to the nearest 0.1 mm. Clam biomass was measured as wet weight, including the shell and flesh dry weight. The flesh dry weight was measured after drying for 48 h at 60 °C. Growth performance was determined as specific growth rate according to the equation $SGR= ((\ln L_t - \ln Lt_0)/(t-t_0)) \times 100$, where $Lt_0$ is the initial length, $L_t$ is the final length, and $t\text{-}t_0$ is the elapsed time in days as in Zanella et al. (2025) using length instead of weight.

## Results

The ranges and means (min-max, mean) of physicochemical parameters during the first experiment were the following, temperature: 5.7 -29.8 °C, 19.2 °C; practical salinity: 22.1-38.3, 35.6; oxygen concentration: 3.0-11.7, 6.3 mg/L; pH: 7.1-8.5, 7.9. During the second experiment, the ranges and means in pond 1 were the following, temperature: 9.7-29.5 °C, 17.6 °C; practical salinity: 32.7-37.5, 35.2; oxygen concentration: 2.3-9.6 mg/L, 6.6 mg/L; pH: 7.6-8.5, 8.1; and temperature: 9.4-29.3, 17.5; practical salinity: 32.4-37.9, 35.1; oxygen concentration: 2.8-9.4 mg/L, 6.5 mg/L; pH: 7.5-8.9, 8 in pond 2.

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Chlorophyll-*a* concentration during the first experiment at the water catchment point in Alfacs Bay was 3.8 ± 1.6 µg/L, while the concentration at the pond inlet was only 0.5 ± 0.1 µg/L (March 2020-February 2021), therefore from March to November 2021 we moved the water catchment point to a small lagoon closer to the experimental pond where chlorophyll-a concentration was higher (1.4 ± 1.6 µg/L). During the second experiment, chlorophyll-*a* concentration at the inlet was 0.8 ± 1.0 µg/L in pond 1 and 0.9 ± 1.1 µg/L in pond 2. Chlorophyll-*a* concentration at the outlet of the ponds was slightly higher, 1.5 ±1.7 µg/L in pond 1 and 1.0 ±1.4 µg/L in pond 2.

Clams growth performance during the period from March 2020 to November 2021 was 0.1 (%/day) starting from a shell length of 16.5 ± 1 mm (mean ± SD) and reaching 29.4 ± 3.1 mm after 618 days; while for the second experiment from November 2024 to August 2025 it was 0.21 in pond 1 and 0.20 (%/day) in pond 2, starting from a shell length 18.7 ± 1.1 mm, reaching 32.7 ± 4.6 mm in pond 1 and 32 ± 3.3 mm in pond 2 after 272 days of growth.

**Discussion and conclusions**

Pond integrated multi-trophic aquaculture (IMTA) has shown to result in improved Manila clam growth performance under similar culture conditions in comparison to Manila clam pond monoculture. Reduced clam seeding density and increased water flow during the IMTA trial may have also contribute to obtain double growth performance in IMTA in comparison to the clam monoculture, but the supply of fish fed together with the microphytobenthos resuspension seem to have contributed to this improved results since chlorophyll-*a* concentration at the outlet of the IMTA ponds was higher than the concentration at the inlet.

**Acknowledgments**

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# EFFECTS OF *Perkinsus olseni* INFECTION IN MICROBIOME COMMUNITY, GENE EXPRESSION RESPONSE AND OVERALL PREVALENCE IN COMMERCIAL CLAM SPECIES

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**Introduction**

The protozoan parasite *Perkinsus olseni* is a global threat to bivalve mollusks, with a deleterious effect in growth performance as well as reproduction capacity[1], including economically valuable species such as *Ruditapes decussatus* (RD), *Ruditapes philippinarum* (RP), and *Venerupis corrugata* (VC), the most economically valuable species of clams in Portugal.

This study was designed to mimic a natural infection and aimed to investigate the response of these three clam species to the parasite. Seed (5-7 mm length) was exposed with two different concentrations of parasite to identify the differential susceptibility of the species to the disease. The gene expression profile at short and long term was determined by RNA-Seq and also the microbiota community composition was evaluated.

**Material and methods**

After isolation of parasite hypnospores[2], two different concentrations were added to tanks (Low Infection (LI) treatment $5x10^5$ cells; High Infection treatment (HI) $5x10^6$ cells). After 48 hours, water was renewed, and the progression of the infection was followed for one month. Samples were taken over four time periods (48 hours, 7-, 14- and 30-days post exposure) to assess short and longer-term responses. Presence and prevalence of parasite over time was evaluated by qPCR[3]. Also, RNA was extracted from samples of 48 hours and 30 days' post exposure. The microbiota community was evaluated by the sequencing of the V3-V4 region of the 16S after isolating the DNA from the whole clam at 30 days' post-exposure in control and HI treatment to verify if the infection had an impact on the community.

**Results**

While in HI treatment all clams became infected, in LI treatment the prevalence indicates a higher predisposition of RD and RP to the disease (97.5% and 76.6% prevalence, respectively), followed by VC (74.4% prevalence). Regarding the parasite load of infected individuals, results showed significant differences in the infection intensity of each clam species to the parasite, with RP showing higher parasite load throughout the experiment. Conversely, VC demonstrated a lower susceptibility to the parasite, with lower parasite load observed at short and long term while RD showed an intermediate response, falling between the other two species (Figure 1).

At transcriptomic level, all species seems to be compromised by the infection at both timepoints, functions such as immune system, reproduction and cellular proliferation were compromised, and just some functions such as dopamine norephrine biogenic amine related to stress responses and regulation of feeding networks were up-regulated[4].

Regarding microbiota community, there is no clear impact on the composition after infection, but some groups seemed to be modulated by the parasite infection. On the other hand, there is a clear separation of the microbiota composition among species (Figure 2).

In conclusion, these findings highlight the variable response of three clam species to *P. olseni*, suggesting that VC may be less susceptible to the parasite, while RP may be the most susceptible. This study provides valuable information for understanding and managing the impact of *P. olseni* on clam populations.

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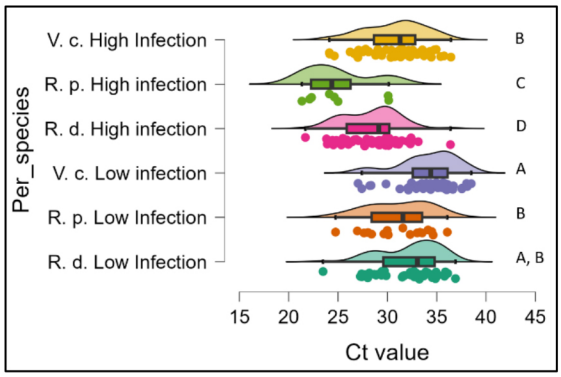


Figure 1. Infection level data between species with low and high parasitic load. Different letters denote statistical differences among groups ($p<0.05$)

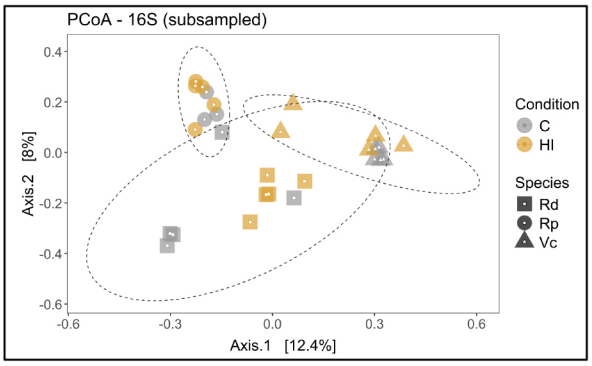


Figure 2. PCoA of the microbiota community at 30 days post-exposure of the three clam species at control and high infection condition.

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# SPECIES SELECTION OF MACROALGAE FOR APPLICATION WITHIN IMTA IN OFF COAST AREAS OF THE MEDITERRANEAN SEA

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## Introduction

Aquaculture has rapidly expanded in the Mediterranean Sea in the last decades, with species like European seabass, gilthead seabream, mussels, oysters, and clams dominating production (FAO, 2024). Concerns about environmental impact, particularly waste deposition, have led to the exploration of integrated multi-trophic aquaculture (IMTA) as a more sustainable approach (COM/2021/236). Thus, the inclusion of macroalgae in IMTA systems has always had strong support from the scientific community. The combination of species present in the area and the opportunities that these entail, bring about a best option for the industry.

The main aim of this study is to overview the outline of adequate macroalgae species with potential for IMTA in the four main geographical areas of the Mediterranean Sea and their value as secondary product such as biometabolite producers.

## Material and Methods

Information used to design this study was obtained from conference papers, indexed journals and concrete literature found in databases such as Science Direct, Google Scholar or Scopus using PRISMA methodology. Subsequently, a SWOT analysis was used to evaluate candidate macroalgae species in the Western, Central and Eastern Mediterranean and Adriatic Basin for their presence and potential use in IMTA systems. The 'Strengths and weaknesses' category in this case include inner traits related to species biology such as culture chances related to natural distributions in waters of a country . The 'Threats' section is defined by specific critical traits that could either endanger or promote deleterious consequences for the environment of the aquaculture production nearby. One of such situations involve the appearance of introduced species or bloom-inducing genera with deleterious consequences for fish production. Finally, the last section was constructed around the interest or commercial potential of the species found ('Opportunities').

*Table 1. Summary of the SWOT analysis for potentially cultivable species of the Western, Central, Eastern Mediterranean, and Adriatic Basin.*

| Region | Promising Species | Cage Presence | Strengths | Weaknesses | Opportunities | Threats | Notes |
|---|---|---|---|---|---|---|---|
| Western Mediterranean | *Ulva rigida, Gracilaria gracilis* | Yes | + | + | ++ | + | High growth, polysaccharides |
| Adriatic Sea | *Ulva lactuca, Chaetomorpha linum Gelidium spp.* | Yes | ++ | + | + | + | Good growth, antioxidant activity |
| Central Mediterranean | Ulva spp. *Gracilaria spp. Chaetomorpha linum* | Likely | ++ | + | ++ | + | Bioactive peptides, rapid growth |
| Eastern Mediterranean | *Ulva fasciata, Gracilaria* sp. | Yes | ++ | + | +++ | + | Protein-rich, IMTA trials support |

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**Results & Discussion**

This study analysed data of 162 algae, categorised into 42 green, 50 brown and 70 red algae. The critical components to be discussed about algae selection for IMTA systems refer to the selection system in the four areas of the Mediterranean Sea. The analysis of threats and opportunities is pertained to the present and even more importantly, also the future environment. Determining the importance of these factors by SWOT analysis gives a clearer view of the chances of success of algae cultures embedded in IMTA production systems. Thus, the selected genera for Western, Central, Eastern Mediterranean, and Adriatic Basin encompass a diverse range of macroalgal species (Table 1).

The species identified in our study craft primary and secondary metabolites, some indispensable for internal growth and metabolism, while others exhibit substantial potential for broader applications in various biological processes. Algal metabolites, spanning polyphenols, alkaloids, terpenes, polysaccharides, fatty acids, sterols, lactones, proteins, and peptides, embody diverse structural and functional classes, each wielding significant biological activities.

The selected macroalgae according to SWOT results belong to genus such as *Chaetomorpha*, *Ulva*, or *Gracilaria,* which are also found among fouling communities, collectively enrich the algal tapestry within aquatic farming facilities. They exhibit unique biochemical characteristics, making them promising subjects for biotechnological exploration.

The convergence of low trophic level species selection and region-specific macroalgae cultivation represents a pioneering step towards a more sustainable and adaptive future for Mediterranean aquaculture. These results provide valuable insights for stakeholders and decision-makers, aiding in implementation of IMTA systems and the sustainable development of aquaculture in the Mediterranean.

**Acknowledgments**

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# TEMPORAL EVOLUTION OF mRNA ABUNDANCE OF APPETITE-REGULATING GENES IN THE GASTROINTESTINAL TRACT AND BRAIN OF RAINBOW TROUT (*Oncorhynchus Mykiss*) FED A PLANT-BASED DIET FROM FIRST FEEDING

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## Introduction

In order to promote sustainable aquaculture practices, fishmeal and fish oil from aquafeeds are being progressively replaced with plant-based alternatives. However, this substitution may negatively affect carnivorous species, such as rainbow trout (*Oncorhynchus mykiss*), especially during early developmental stages (Lazzarotto et al., 2018). The underlying molecular mechanisms of these effects are not yet fully understood. To shed light on this, the aim of this study was to characterize the temporal evolution of the mRNA abundance of amino acid sensing systems in the gastrointestinal tract (GIT) and of appetite-regulatory genes in the brain of rainbow trout from the first feeding, as well as to evaluate the putative modulation of such temporal profiles of a plant-based diet.

## Materials and methods

Rainbow trout juveniles were fed two experimental diets from the first feeding until 100 days: a control diet similar to a commercial feed (containing a mix of fishmeal and plant meal), and a 100 % plant-based diet. Samples of the GIT and brain were collected on days 0 (before the first meal) and 2, 5, 10, 30, and 100 post-initiation of feeding. The abundance of genes related to amino acid sensing and appetite-regulatory hormones in the GIT and to feed intake regulation in the brain was quantified by quantitative PCR (qPCR).

## Results and discussion

Trout fed the plant-based diet exhibited lower feed intake levels compared to those fed the commercial-like diet, resulting in reduced final growth. This difference may be attributed to a lower palatability of the plant-based diet, possibly due to anti-nutritional factors or differences in nutrient composition, or to a putative alteration of nutrient sensing mechanisms and feed motivation, ultimately affecting central regulation in the long term.

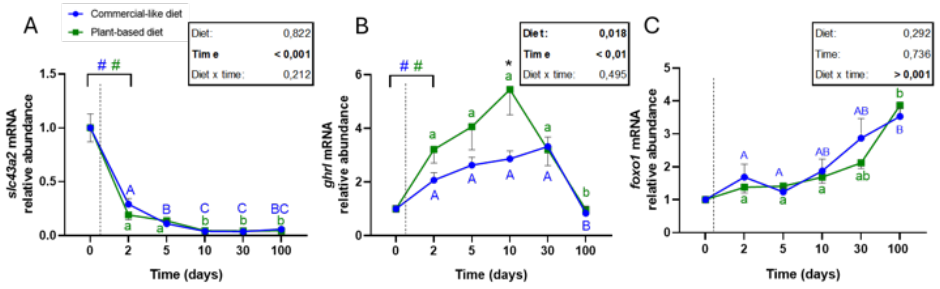


Figure 1. Temporal expression patterns of three of the studied genes in the GIT (A and B) and brain (C) of rainbow trout fed with either a commercial-like or a plant-based diet diets during the first months of life. Figure shows the relative mRNA abundance before the first feeding (time 0) until 100 days after being fed. The significant effect of time ($P < 0.05$) within each dietary condition is indicated by uppercase blue letters (commercial-like diet) and lowercase green letters (plant-based diet), while asterisks (*) denote significant differences ($P < 0.05$) between the two diets at a given time (two-way ANOVA). Pound symbol denote significant differences ($P < 0.05$) between day 0 and day 2 (Student's t-test).

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Regarding amino acid sensing mechanisms, the commercial-like diet induced an increase in the mRNA abundance of one of the studied receptors (*tas1r2a*) at the onset of feeding, although no major changes were then observed throughout development. As for amino acid transporters, the first feeding with either a commercial-like or plant-based diet modulated the mRNA abundance of *slc43a2* and *slc1a5*. Subsequently, increasing mRNA abundance over time was observed for *slc6a19*, *slc15a1*, and *slc1a1*, while decreasing mRNA levels at early stages were found for *slc7a9* and *slc43a2* (Fig. 1A). No major differences in the temporal mRNA abundance profile were observed between diets (except for *slc1a5*).

The onset of feeding (with one or both diets) caused a significant increase in the mRNA levels of almost all gastrointestinal hormones (*ghrl*, *gcg*, *pyy*) (Fig. 1B). At early developmental stages, an increase in mRNA abundance was observed for all hormones, which may reflect the need to regulate feeding through the overexpression of appetite-regulating hormones. However, a decrease in abundance was observed at late stages, which could be attributed to more precise hormonal regulation due to the maturation of these mechanisms.

At the central level, all neuropeptides exhibited significant changes at the onset of feeding, with one or both diets. After the initial feeding, *npy* and *cartpt* (particularly with the control diet) increased progressively throughout development, whereas *agrp* and *pomc* showed a decreasing trend over time. Additionally, the expression of key components of integrative signalling pathways (*prkaa2*, *mtor*, *creb1*, *foxo1*) increased during growth (Fig. 1C), although their abundance was differentially modulated depending on the dietary treatment. Furthermore, no major differences between diets were observed, except for *mtor*.

**Conclusions**

This study provides the first description of the mRNA abundance of appetite-regulating mechanisms in the GIT and brain of rainbow trout from first feeding up to 100 days. The results indicate distinct temporal gene expression patterns in both tissues, with almost no significant dietary (commercial-like *vs*. plant-based diet) effects during early developmental stages. This suggests that reduced feed intake and growth impairments associated with vegetable diets may be due to other physiological mechanisms. These findings are essential for optimizing feeding strategies in sustainable aquaculture.

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# EFFECTS OF HIGH-LEVEL FISHMEAL REPLACEMENT ON GROWTH, GUT AND BIOFLOC MICROBIOTA, AND POST-HARVEST QUALITY EVALUATION IN *Penaeus vannamei* UNDER BFT

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## Introduction

Fishmeal (FM) is the main cost driver in shrimp aquaculture, accounting for 50–70% of total production costs. Previous studies have demonstrated that *Penaeus vannamei* can tolerate partial FM replacement without compromising growth performance. Additionally, biofloc technology (BFT) provides a nutritional supplement and enhances shrimp immunity. This study aimed to evaluate the impact of FM substitution with alternative protein sources in shrimp diets on the microbial composition of biofloc, and to explore potentials interaction with intestinal microbiota, growth performance and product quality.

## Material and Methods

Six experimental diets were tested in triplicate, featuring either 92% or 100% replacement of fishmeal (FM) with alternative protein sources of animal origin (A92, A100), plant origin (P92, P100), or a combination of both (AP92, AP100). All diets were isoproteic (38% protein) and isolipidic (10% fat). Shrimp were cultured in 1 $m^3$ tanks at 350 individuals/$m^3$ from 2.5 g to 8 g average weight. Growth was monitored biweekly. Biofloc and shrimp gut microbiota were characterized by full-length 16S rRNA gene sequencing (PacBio Sequel II), and amplicon sequence variants were analyzed using QIIME2 and CLR transformation. Sensory quality of shrimp from selected diets was evaluated at 1 and 7 days post-slaughter following ISO standards.

## Results and discussion

Water quality parameters remained within optimal ranges for shrimp culture. Growth performance confirmed that up to 92% FM replacement with animal (A92) and mix of animal/plant protein sources (AP92) is feasible without negative effects. These findings exceed the FM substitution levels reported in previous studies using plant- or animal-based alternatives, or their combination (Aya et al., 2015, Cheng et al., 2002, Hulefeld et al. 2018).

High-quality 16S rRNA gene sequencing data were obtained across all treatments, with a minimum of 81% non-chimeric reads per sample. The most abundant phyla in biofloc bacterial community were *Proteobacteria*, *Patescibacteria,* and *Planctomycetota,* which together represented approximately 68% of the total microbial community. At the taxonomic level of order, *SBR1031* and *Pirellulales* were dominant, accounting for 29% of the overall composition. At the family level, the most prevalent taxa were *Woesebacteria* (14%) and *Pirellulaceae* (12%; (Figure 1A). While the presence of *Proteobacteria* and *Planctomycetota* is commonly reported in biofloc systems (Tanay et al. 2020, Vinasyiam et al. 2025), the detection of *Patescibacteria*, although possible, is often associated with methanogenic archaea. This finding may suggest the formation of microenvironments with reduced oxygen availability within the culture system (Kuroda et al. 2024). Furthermore, the presence of *Woesebacteria* is associated with anoxygenic denitrification (Rajeev et al 2024), and the *Pirellulales* and *Pirellulaceae,* taxa are typically found in low-oxygen environments (Dedysh et al. 2020), supports this hypothesis. In contrast, the intestinal microbiota of shrimp was primarily composed of *Proteobacteria, Planctomycetota, Chloroflexi,* and Actinobacteriota, which together constituted 88% of the microbial community. This profile is consistent with previously reported profiles in shrimp of similar size classes (Zeng et al. 2017). At both the order and family levels, *Pirellulales* and the corresponding *Pirellulaceae* were the most abundant taxa, each representing approximately 18% of the total microbiota (Figure 1B). The composition of the shrimp gut microbiome is known to be strongly influenced by environmental parameters, farming conditions, and the developmental stage of the animals. The consistent dominance of *Pirellula* at both the order and family levels reflects this influence. Moreover, the occurrence of the main phyla is consistent with previously reported profiles in shrimp of similar size classes (Zeng et al. 2017).

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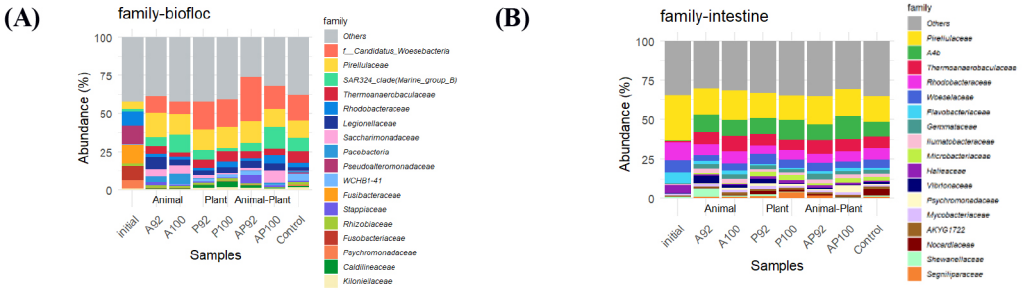


Figure 1: Family taxon level relative abundance at the begging of the experiment (initial) and at the end by treatment (A92, A100, P92, P100, AP92, AP100, Control), evaluated at biofloc (A) and shrimp intestine (B) samples.

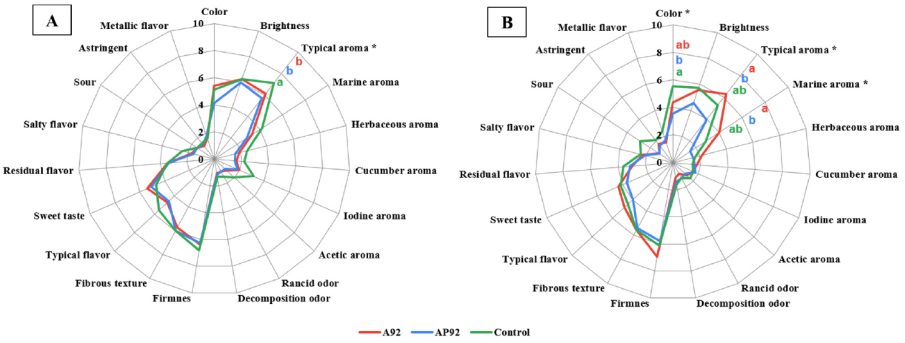


Figure 2: Sensory profile of shrimp fed three diets (A92, AP92, and Control), evaluated at one- (A) and seven- (B) days post-slaughter. (*) symbol represents significant difference between treatments ($p<0.05$).

Regarding alpha diversity, analysis revealed a lower microbial diversity in the initial shrimp samples, based on the Simpson and Shannon indices. However, no significant differences were found at the end of the trial between treatments in either biofloc or shrimp samples. In terms of beta diversity, no statistically significant differences were detected among treatments in any samples.

In relation to the sensory profiles, by day 1, a distinctive shrimp aroma was observed to the Control treatment. Meanwhile by day 7, Control shrimp exhibited the highest color scores, while A92 shrimp obtained the highest marine a typical aroma score (Figure 2. A y B). The melanosis incidence averaged 9% across all treatments initially. However, by day 7, shrimp fed the AP92 diet showed significantly lower melanosis levels (25%) compared to other groups, indicating reduced susceptibility associated with this dietary treatment.

In summary, up to 92% of fishmeal can be replaced with animal or mixed alternative proteins without compromising shrimp growth, water quality, microbiota stability, or sensory quality. Diet composition influenced shrimp-associated microbial communities more than biofloc communities, suggesting host-specific filtering mechanisms. In addition, certain alternative protein diets (AP92) may improve post-harvest quality by reducing melanosis, highlighting the potential of these formulations for sustainable shrimp production.

**Acknowledgments**

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# DECODING THE BIOFLOC BLUEPRINT: METAGENOMIC INSIGHTS INTO THE RESHAPING OF AQUACULTURE MICROBIOMES

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## Introduction

The incorporation of microalgae and bacterial probiotics into Biofloc Technology (BFT) systems has received growing attention in recent studies focused on *Penaeus vannamei* culture. Supplementation with *Chlorella vulgaris* has been associated with improvements in water quality, growth performance, and overall shrimp health (Swapna et al. 2015). The present study aims to evaluate the potential benefits of such supplementation by characterizing the microbial community structure through metagenomic analysis. Special emphasis is placed on biofloc systems enriched with *C. vulgaris* and a commercial probiotic (*Sanolife PRO-W*, INVE).

## Material and Methods

Two treatments were evaluated (MicroProb) consisting of a biofloc system supplemented with *C. vulgaris* at a concentration of $7 \times 10^6$ cells/mL and an initial dose of a commercial probiotic (*Sanolife PRO-W*, INVE) at 3 g/m³, followed by weekly maintenance doses of 1 g/m³, maintained under a 12:12h light:dark photoperiod; and (Control) a non-supplemented biofloc system maintained under continuous darkness (0:24h photoperiod). The microalgae culture was maintained at 28 °C and 21 PSU salinity, with continuous aeration and illumination ranging from 3,500 to 4,000 lux. The probiotic consisted mainly of *Bacillus subtilis* and *Bacillus licheniformis* spores at a concentration of $5 \times 10^{10}$ CFU/g. *P. vannamei* ($\rho$= 205 animals/m²) were fed twice per day with a commercial feed (LeGoussant). Water quality parameters were maintained within optimal ranges for white shrimp (Van Wyk, 1999) and growth was assessed biweekly via random weight sampling (Kuhn et al., 2010). Biofloc samples were collected at the end of the experiment to ensure representative collection. Shotgun metagenomic sequencing using the Illumina NextSeq 2000 platform. Assembly and binning workflows (metaSPAdes and MaxBin2) enabled reconstruction of metagenome-assembled genomes (MAGs), which were taxonomically classified with GTDB-Tk and functionally annotated via Prokka and DRAM.

## Results and discussion

Water quality parameters remained within optimal levels for white shrimp culture. Shrimp growth performance showed no significant differences between treatments. In the metagenomic analysis, bacteria, archaea, virus, and eukaryotic organisms were identified from the raw reads. However, the MAGs assembly was limited to bacteria and archaea, constraining subsequent analysis. A total of 205 and 210 non-redundant MAGs were recovered from the MicroProb and Control treatments, respectively, with similar proportions of bacterial and archaeal genomes (198 bacterial and 7 archaeal MAGs in MicroProb; 204 bacterial and 6 archaeal in Control). Analysis revealed 26 MAGs exclusive to MicroProb, 28 exclusive to Control, and 19 shared across both treatments. MAG quality metrics, including completeness and contamination, were comparable between treatments. However, MicroProb showed a greater concentration of genomes with intermediate sizes (4–5 Mbp) (Figure 1A) and gene counts (3,000–6,000 genes) (Figure 1B), suggesting a more uniform and potentially functionally balanced microbial community. In contrast, the Control treatment showed a greater number of highly fragmented MAGs and genomes with extreme sizes.

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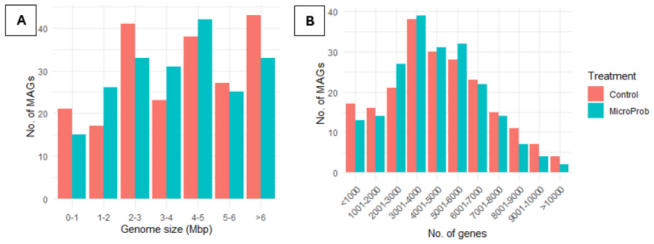


Figure 1: Genome size (A) and number of genes (B) per number of MAGs recovered in each treatment (Red: Control, Blue: MicroProb).

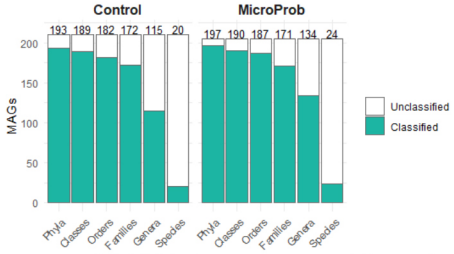


Figure 2: Number of recovered MAGs by treatment (Control, and MicroProb) based taxonomic level classification.

Taxonomic classification was highly effective at higher taxonomic levels, successfully assigning approximately 89% of MAGs to the family level in both treatments. A total of 30 phyla, 56 classes, 91 orders, 118 families, and 24 species were identified. However, a substantial proportion of MAGs remained unclassified at the genus and species levels, indicating the presence of potentially novel or poorly characterized taxa (Figure 2). Dominant bacterial groups shared across both treatments included *Pseudomonadota*, *Bacteroidota*, and *Planctomycetota* (phylum level), *Gammaproteobacteria* (class), and *Pseudomonadales* (order), which are commonly reported in BFT systems (Baliga et al., 2021; Ghonomy et al., 2023). Notably, MicroProb displayed a higher abundance of families such as *Halieaceae*, *Saprospiraceae*, and *Woeseiaceae* (>5 MAGs), which were present only at low abundance in Control. These families have been linked to key ecosystem functions in BFT: *Halieaceae* and *Saprospiraceae* contribute to ammonium removal, while *Woeseiaceae* has been associated with anaerobic denitrification, enhancing nutrient recycling and system sustainability (Rajeev et al., 2024).

Overall, shotgun metagenomic profiling suggests that biofloc supplementation with microalgae and probiotics selectively enriched beneficial and functionally relevant microbial taxa. These changes support the hypothesis that targeted additives can restructure biofloc microbial communities, promoting ecological functions favorable for water quality and shrimp growth performance. A multikingdom analysis will be performed based on the metatranscriptomic assembly, enabling the identification and quantification of a wide range of eukaryotic organisms present in the biofloc.

**Acknowledgments**

This work was supported by European Union Next Generation-Plan de Recuperación-Ministerio de Ciencia e Innovación-Gobierno de España (TED2021-129272B-C21). T. Cascales contract was supported by European Union Next Generation-Plan of Conselleria d'innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434), and Research Personnel Training Grant (PRE2021-098367); supported by MCIN/ AEI/10.13039/501100011033/.

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# KEEPING IT FRESH: HOW SLAUGHTER METHODS IMPACT QUALITY AND SHELF LIFE OF BIOFLOC-CULTURED SHRIMP

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## Introduction

Shrimp are a highly perishable product due to their rapid microbiological and biochemical spoilage post-harvest. The biofloc system contains a rich microbial community that benefits *Penaeus vannamei* production, modulates its associated gut microbiota, and enhances the immune system, potentially influencing post-mortem freshness. Ensuring high product quality is essential for market acceptance, and the initial microbial load plays a key role in shelf life. This study evaluates the impact of different slaughter methods on the freshness and refrigerated stability of shrimp reared under biofloc conditions, highlighting the interaction between microbiota, slaughter technique, and quality parameters as a basis for optimizing post-harvest practices in intensive aquaculture.

## Material and Methods

Shrimp were cultured at 350 kg/m³ in 3 m³ indoor biofloc tanks and fed commercial feed (LeGoussant) until reaching average weight of 15 g. Water quality parameters were maintained within optimal ranges for white shrimp (Van Wyk, 1999). Four slaughter methods were evaluated: cold thermal shock using refrigerated seawater (-2ºC) (TS, control) (TS was implemented in all slaughter methods); 5 min immersion in refrigerated seawater (0±2.5 ºC) with 50 ppm bleach followed by TS (5BTS); clarification (3–4 h) before 50 ppm bleach and TS (C1BTS); and TS followed by 2 min UV exposure in each side of the shrimp (TSUV). After slaughter, shrimp were packed in sealed plastic bags and cold storage icing was performed over time at 0±2 ºC between ice blocks. A shelf-life study was conducted at 0-, 3-, 6-, 9-, and 12-days *post-mortem*; microbiological count, physicochemical parameters (TVB-N) and Quantitative Descriptive Analysis (QDA) analyses were performed according to the standard ISO 13299 (Sensory profile).

## Results and discussion

Microbiological analysis during cold storage of shrimp revealed significant temporal changes across all bacterial groups. Mesophilic bacteria remained most stable in the C1BTS treatment (~5.2 log CFU/g), likely due to gut-emptying prior to slaughter, while other treatments (TS, 5BTS, TSUV) began with higher counts and declined over time. Enterobacteria increased by ~3 log CFU/g by day 9 before returning to baseline, aligning with previous studies. Pseudomonas spp. declined until day 9, particularly in TS, then slightly rose by day 12, consistent with cold-shock recovery patterns. Spoilage-specific organisms decreased steadily, with initial higher counts in TS and TSUV. Psychrotrophic bacteria increased most markedly in TS, 5BTS, and TSUV (>2 log CFU/g), peaking at ~5.3 log CFU/g, while remaining lowest (~3.5 log CFU/g) in C1BTS. C1BTS consistently demonstrated superior microbial control. Total Volatile Basic Nitrogen (TVB-N) levels, an indicator of spoilage, increased across treatments but remained significantly lower in C1BTS. A strong correlation ($r = 0.987$) between psychrotrophic bacteria and TVB-N suggests that C1BTS reduced microbial growth and enzymatic degradation, thus improving shelf-life.

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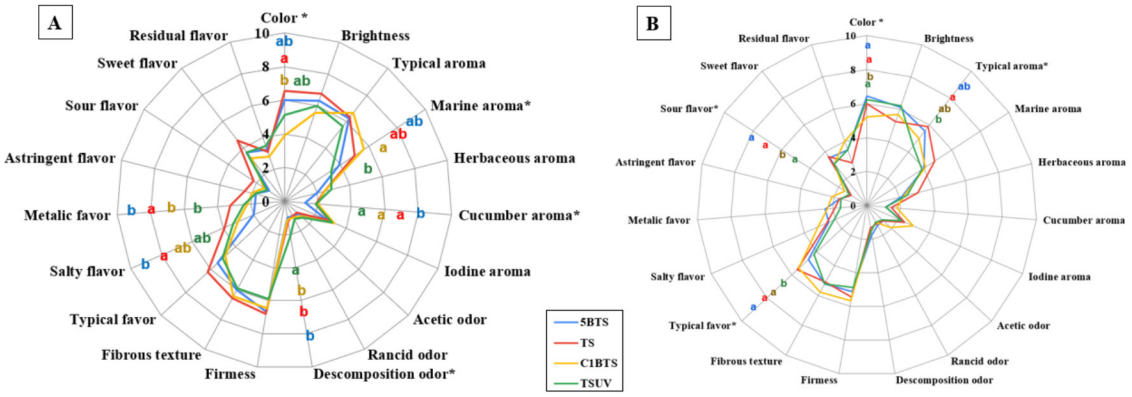


Figure 1: Sensory profile of shrimp slaughtered by 4 methodologies tested (TS, C1BTS, C5BTS and TSUV) * significant differences Letters Homogenius group

QDA analysis was used to assess the sensory quality of shrimp at 1- and 7-day post-mortem. Evaluations were conducted in the tasting room of the Pilot Plant, compliant with ISO 8589:2007.

Shrimp were served whole, boiled, and at room temperature to pre-trained evaluators. The sensory descriptors were established prior to the sessions. Over time, significant freshness-related descriptors such as marine and cucumber aroma, metallic and salty flavor (day 1) disappeared in all treatments, and were replaced by descriptors like typical aroma, typical flavor, and sour flavor (day 7). By treatment, TS stood out for their color, cucumber aroma, and metallic and salty flavors on day 1. And maintained superior scores in typical aroma and flavor and had the lowest sourness by day 7. C1BTS shrimp showed strong marine aroma at day 1 but poor color, which persisted through day 7. Additionally, these samples exhibited the highest sourness on day 7. The 5BTS shrimp with consistently intermediate profiles across all descriptors. TSUV group performed worst overall. They scored lowest in marine and typical aromas and highest in spoilage odor (day 1) and sourness (day 7), suggesting accelerated sensory degradation.

Overall, the C1BTS treatment proved to be the most effective in preserving shrimp quality during refrigerated storage, as evidenced by lower microbial loads, reduced spoilage indicators, and stable organoleptic attributes throughout the study. These findings underscore the potential of C1BTS as a promising approach to extend the shelf life of fresh shrimp under cold chain conditions.

## Acknowledgments

This work was supported by I+D+i Research Project: “Pursuing the zero waste in Biofloc Technology to produce a sustainable and biosafe aquaculture product (ZeroFloc)” (PID2023-149570OB-I00). S.Ferrando-Juan has a predoctoral Research Personnel Training Grant (PRE2021-098367); supported by MCIN/ AEI/10.13039/501100011033/. J. Gómez-Aguilera contract was supported by European Union Next Generation-Plan of Conselleria d’innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434).

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# IMMUNOMODULATORY EFFECTS OF *Salicornia ramosissima* EXTRACTS ON ATLANTIC SALMON ADHERENT LEUKOCYTES: TIME-DEPENDENT FUNCTIONAL AND TRANSCRIPTOMIC RESPONSES

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**Introduction**

The growing demand to adhere to sustainable practices in aquaculture has driven interest in plant-derived immunostimulants. Halophytes, such as *Salicornia ramosissima*, are rich in bioactive compounds that may have immunomodulatory properties and could benefit farmed fish. This study investigated the effects of several *S. ramosissima* extracts on head kidney primary adherent leukocytes from Atlantic salmon (*Salmo salar*), focusing on innate immune responses at both functional and molecular levels.

**Materials and Methods**

Head kidney primary cells were harvested from healthy Atlantic salmon post-smolts maintained at the Research Station of Nord University. Adherent leukocytes, isolated using established protocols, were exposed to five different types of *Salicornia ramosissima* extracts (CAS.EX. 1-5), derived from distinct steps of the extraction cascade. Cytotoxicity was assessed using an MTT assay, and non-toxic concentrations were identified. Adherent leukocytes were exposed to the non-toxic concentration of each extract for either 3 or 24 hours, followed by a challenge with inactivated *Tenacibaculum maritimum*. Phagocytosis and ROS production were measured using Imaging Flow Cytometry. Furthermore, a snapshot of the changes in the transcriptome of the leukocytes was captured to reveal the responses at the molecular level.

**Results and Discussion**

Exposure to CAS.EX. 4 significantly enhanced phagocytic activity and ROS production after 24 h. Transcriptomic analysis revealed distinct early and late-stage immune responses. Within 3 h, CAS.EX. 4 triggered changes in nucleocytoplasmic transport, lysosomal function and lysine degradation. Extending the incubation time to 24 h, activated immune and stress-related pathways, including proteasome function, phagosome formation and autophagy while suppressing cell cycle and DNA repair pathways. These findings suggest that CAS.EX. 4 acts as a potent immunostimulant, enhancing phagocytic activity, stress adaptation, and cellular homeostasis mechanisms in adherent leukocytes from Atlantic salmon. The observed immune modulation and functional outcomes support the potential of *Salicornia ramosissima* extract as a functional ingredient to promote fish health in aquaculture.

**Acknowledgments**

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# THE FINS MODEL—A WEB-BASED FRAMEWORK FOR AQUACULTURE SUSTAINABILITY

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## Introduction

The supply of aquatic products for human food predominantly relies on aquaculture, which is now 57% of global production (FAO, 2024). In recent years, annual growth has been 6.6%, but in the West growth has been considerably slower or flatlined.

Fig. 1 shows that growth in Europe (data from Eurostat: http://meta.longline.co.uk) was mainly driven by Norway (salmon) and Turkey (bass and bream). Even if the UK is excluded due to Brexit, the EU APR for the period is -0.1%, compared with 7.9% for non-EU European nations (9% if the UK is excluded from the calculations).

North America is in a similar situation, with a 2008-2022 APR of 0.5% for Canada and 2.3% for the USA. The total production for Europe and North America in 2022 is 4.2 million tonnes, 3% of world production, for 12% of the world population. In Europe, demand for seafood has remained constant, with 22.2 kg per capita $y^{-1}$ in both 2007 and 2024 (FAO, 2010; FAO, 2024) but in Asia, the demand increased from 18.5 kg per capita $y^{-1}$ in 2007 (FAO, 2010) to 24.7 kg per capita $y^{-1}$ in 2024 (FAO, 2024). These numbers are very significant because (i) per capita consumption in Asia is now 11% higher than in Europe, reflecting the increase in Asian per capita GDP; and (ii) Due to the combination of population ratio (Asia : Europe = 6.5) and per capita consumption difference, Asia annually consumes 119 Mt of aquatic products and Europe consumes only 16 Mt. This huge and widening gap, allied to the fact that 90% of aquaculture production occurs in Asia, means that Europe and North America face a critical problem with respect to the supply of aquatic products—and therefore food security for the coming generations.

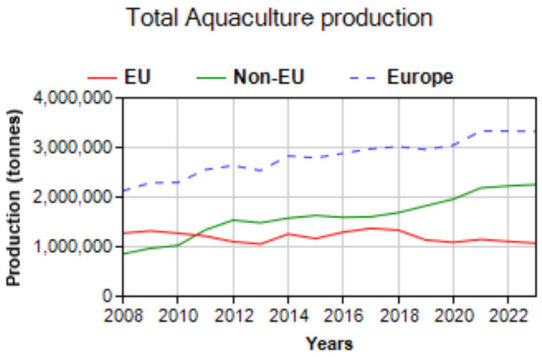


*Fig. 1. European aquaculture production over the last two decades. Growth in the EU has been negative.*

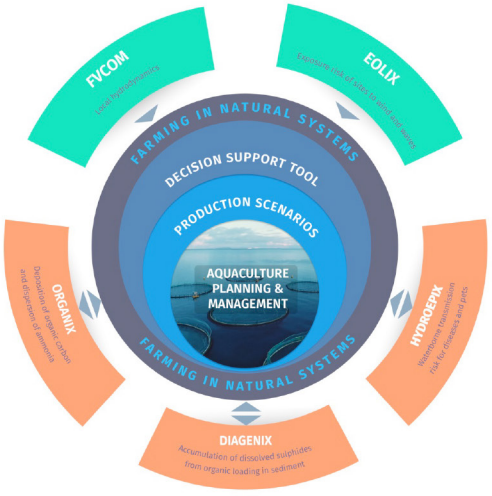


*Fig. 2. FINS framework for aquaculture sustainability and growth.*

Whereas in Asia, freshwater aquaculture in ponds and lakes makes up 70% of production, in the West, cultivation is mainly in coastal bays and estuaries. This means that Marine Spatial Planning (MSP) plays a key role in harmonising multiple uses through appropriate allocation of space (Strand et al, 2025) but MSP determines the effect of the environment on aquaculture—imposing constraints on where the activity can occur; MSP does not address the effect of aquaculture on the environment: other types of mathematical models are required to address this (Ferreira, 2025).

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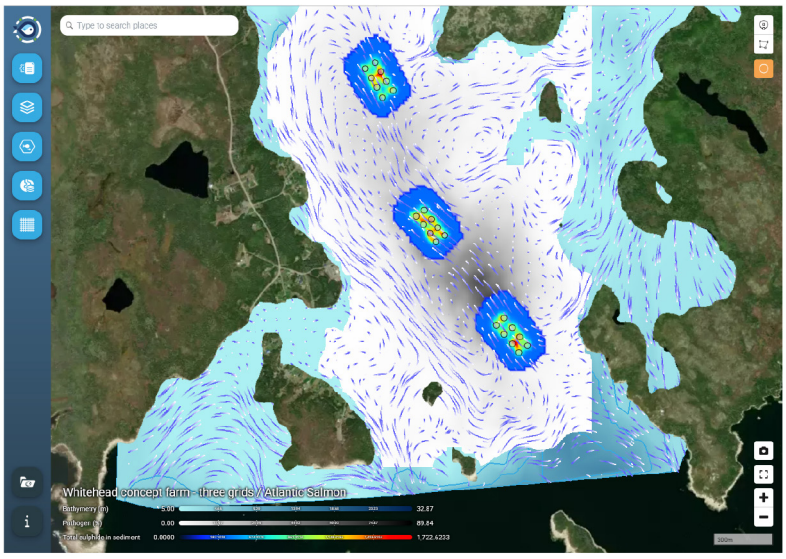


*Fig. 3. FINS results for Whitehead Harbour, Nova Scotia, showing 3 salmon grids, bathymetry (light blue) and velocity fields, sulphide in the sediment (blue-red scale), and pathogen emission from the southernmost grid (greyscale).*

These models are extensively published in the literature and have been applied in various parts of the world, but there is a need for frameworks that bring together complex models to deal with issues such as sediment organic enrichment and pathogen outbreaks, that occur at very different time and space scales.

**Methods**

The FINS (Farming In Natural Systems) framework (Fig. 2) combines a set of models that address specific issues related to finfish and shellfish aquaculture.

These include near-field deposition and fate (diagenesis) of particulate organics, far-field nutrient enrichment, using ammonia as an indicator, together with oxygen reduction, and for bivalves, chlorophyll (as a proxy for food) depletion. Detailed models for water circulation are the base for understanding the distribution of key performance indicators (KPI) of aquaculture sustainability.Associated with these circulation models are well-tested physiological models for growth of finfish and shellfish species such as salmon, bass, bream, oysters, and mussels. FINS is fully georeferenced and can represent multiple types of aquaculture structures within a bay, including cages, cage grids, rafts, and longlines—the location, type, and dimensions of structures are user-defined.

**Results and Discussion**

The results presented in this paper illustrate the outputs of FINS for different KPI in selected bays in Nova Scotia, Canada. Fig. 3 shows four (concept) layers for Whitehead Harbour, Nova Scotia: the bathymetry and residual velocity (from the FVCOM model) for the entire bay and three user-defined grids of eight 30 m diameter salmon cages; outputs of three models (circulation, diagenesis, and pathogen emission) show the effects of salmon cultivation through a 500-day cycle.

The near-field deposition of particulate organics from uneaten and waste feed is simulated, together with the consequences for sediment diagenesis, in this case for sediment sulphide concentration; far-field dispersal of a pathogen emitted from the southernmost grid is also shown—the residual currents transport the pathogen NW leading to infection in the central and northern grids. This allows managers to examine the interaction between existing or potential farms with respect to disease connectivity, promoting improved animal welfare, natural biodiversity, and ecosystem sustainability.

FINS allows for models external to the application to be displayed, including GeoJSON outputs from Geographical Information Systems. This helps to take spatial planning to the next level. By applying different mathematical models to aquaculture structures placed in suitable areas and examining e.g. the potential effect of pathogen dispersal or eutrophication in adjacent zones, MSP can be leveraged to account for the dynamic nature of coastal ecosystems.

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FINS also represents a major technological advance in a platform of this type when compared to conventional desktop solutions. FINS runs in the browser, like any website, and users can access and use it anywhere, from any computer or tablet—even on TV. In a world that increasingly relies on the Internet of Things (IoT), this is a critical advantage. The option to use the GPU for the graphics primitives in FINS, including design of any type of polygon as an aquaculture structure, animation of residual current flows, and various other features, has led to very significant improvements in speed and functionality.

**Acknowledgements**

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# AQRATE—AN INTEGRATED MODEL FOR QUANTIFYING AQUACULTURE CARRYING CAPACITY IN MARINE AND FRESHWATER SYSTEMS

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## Introduction

Aquatic food security is increasingly dependent on farming and accounts for over 57% of all finfish and shellfish consumed worldwide (FAO, 2024). The overwhelming majority of aquaculture takes place in emerging economies (88% of the 131 X $10^6$ t annual production[1] in 2022 (FAO, 2024)), which are largely responsible for the consistent 6-8% annual increase in production. This growth rate, fuelled by the twin pressures of human population expansion and the need to increase per capita consumption of fish, and stimulated by the stagnation or decrease in wild capture for key species, is a clear environmental and social pressure (Costello et al, 2020).

To ensure the sustainable growth of aquaculture, given limited resources such as space and water quality and the need to balance competing uses and equilibrium of ecological, economic, and social factors, it is essential to determine carrying capacity (CC) in an integrated manner (Inglis et al, 2000). CC is presently defined through four pillars: physical, production, ecological, and social (Ferreira et al, 2013; Sanchez-Jerez et al, 2016). Together, these correspond to the FAO definition of the Ecosystem Approach to Aquaculture (EAA), predicated on optimisation and preservation of human capital, ecosystem services, and multiple system uses (Soto et al, 2008).

Although these pillars are accepted as the basis for quantifying sustainable carrying capacity, and different approaches have been proposed to evaluate each component, the pillars need to be combined into a single recommendation to policymakers to provide a number or range for stocking a particular area with cultivated species. To address this need, the Aquaculture Rating Thresholds for Ecosustainability (AQRATE) framework was developed, which brings together the physical, production, ecological, and social pillars of carrying capacity in order to provide a precautionary estimate of where, what, and how much can be cultivated in a given area (Ferreira, 2025). The focus is on open water bodies (inshore or offshore, marine or freshwater), since these present the greatest challenges with respect to connectivity, competing uses, and ecological balance, but a subset of this approach could be applied to pond-based systems.

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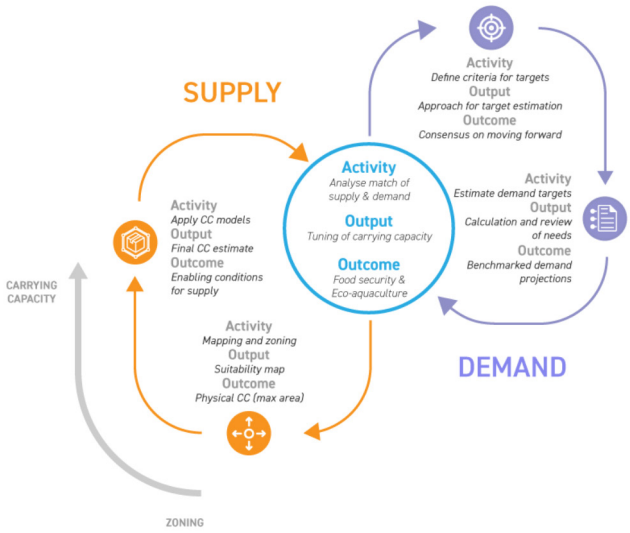


*Fig. 1. General approach to integrated CC assessment.*

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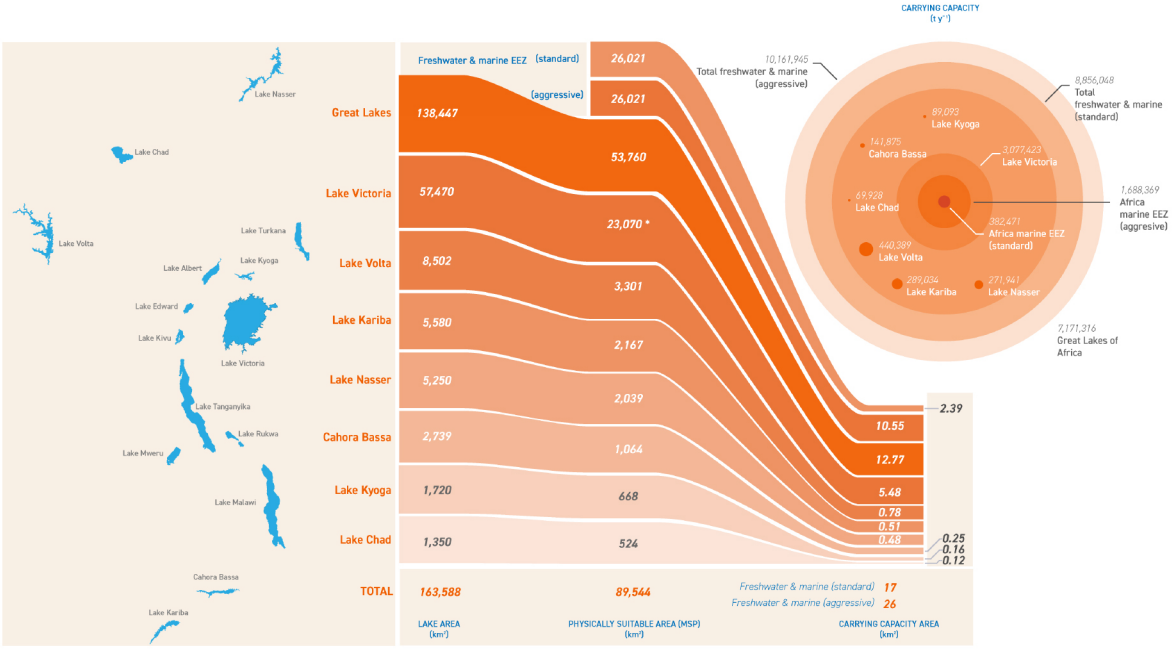


*Suitable inshore area: 3,224 km$^2$; suitable offshore area: 19,846 km$^2$. Total suitable area calculated bottom-up from MSP, all others calculated top-down using the proportion of total area of the system(s).

*Fig. 2. Carrying capacity of the great lakes of Africa and the African marine Exclusive Economic Zones.*

The aims of this work were to:

1. Develop a methodology to integrate different aspects of aquaculture carrying capacity to determine an overall range of areal occupation and stocking density that can be used for management;
2. Test the integrated framework in different ecosystems to make real-world predictions and assess the comparative role of each pillar;
3. Provide policymakers and managers with a framework that is simple to use and only moderately data-intensive, to meet the needs of aquaculture development in different parts of the world.

**Approach**

The general approach used for estimation of carrying capacity is shown in **Error! Reference source not found.**. The supply-side steps (left), address both the MSP process and the determination of carrying capacity. The final value or range for CC corresponds to what can be produced in a sustainable manner. In many parts of the world, inshore aquaculture is prevalent, so the AQRATE framework deals with both inshore and offshore conditions[2].

The main emphasis of this paper is on the development of the framework and therefore the focus is on environmental and social carrying capacity. However, it is important from a policy perspective to understand the potential demand for what can be supplied, to avoid creating unrealistic expectations that can result in negative social and economic consequences.

The output of Marine Spatial Planning (MSP) is the zoning of a particular water body, using a classification scheme normally based on Multi-Criteria Evaluation (MCE) implemented by means of a Geographic Information System (GIS). This broadly corresponds to the physical carrying capacity pillar. MSP yields a set of areas $A_m$ (one per zone) corresponding to the zoning of the water body of interest. To each area $A_m$ is associated a suitability class; therefore, although the sum of zone areas equals the area of the water body, the sum of suitable zone areas will almost always be less than the water body area. For any suitable zone, given the available area $A_m$ determined by MSP, the next step is to determine a precautionary coefficient to be applied, resulting in a new, smaller area $A_{cc}$ that is available for cultivation taking into account the production, ecological, and social pillars.

The precautionary factor a is determined heuristically, taking into account a number of key factors (Eq. 1).

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Where:
$a_r$ = production-based maximization of harvestable biomass (Bacher et al., 1998; Smaal et al., 1998; Inglis et al, 2000), or profit (Ferreira et al, 2007)
$a_e$ = eutrophication potential based on overall dissolved emissions from an area
$a_d$ = pathogen risk and connectivity
$a_s$ = acceptable social impact of structures

Simple zone-scale models are used to quantify the various components of a, including (i) a model for space allocation constraints, such as typical grid layouts and spacing, how many grids should makeup a typical farm, and how far apart farms should be spaced; (ii) a mass balance model for emissions of dissolved nutrients, allowing water quality thresholds to be tested in order to optimise aquaculture stocking density; and (iii) a pathogen model addressing stocking density, connectivity, and risk, which provides information on both stocking density and spatial distribution within an (MSP) suitable area. The final value or range for CC for each zone is determined through a stepwise procedure whereby the lowest estimate for CC is taken to be the recommended value, based on the results obtained from the physical, production, ecological, and social pillars. This assessment must be carried out zone by zone, because the limiting factor can vary among zones (e.g. in some zones eutrophication may limit CC, in others social CC or disease risk may be limiting) and a bulk approach can more than double the estimate of CC, particularly when a simulation considers offshore areas with larger cages and inshore areas with artisanal grids of small cages.

This is compared with the predicted *additional* demand, calculated by comparing current consumption with a policy target. Current consumption already accounts for the mass balance of fisheries and aquaculture and of imports and exports. The comparison between supply and demand can be used to determine how aggressively some of the parameters in AQRATE can be adjusted, e.g. accepting a higher degree of eutrophication or pathogen risk as a trade-off for increasing carrying capacity.

**Results and Discussion**
Two case studies illustrate the application of AQRATE under different conditions. The Kenyan part of Lake Victoria was used for Nile tilapia (*Oreochromis niloticus*) in freshwater, and the offshore coastal area of Pakistan was used to analyse CC for barramundi (*Lates calcarifer*). The approach was then used to estimate the carrying capacity for the whole of Africa. Due to space limitations, only a summary of the results for Kenya and the wider application to Africa are shown herein.

The current population of Kenya is 53.8 million. The estimated CC would be 258,279 t y-1 live weight with the data used in this case study, yielding 180,795 t y-1 of tilapia fillet after deducting 30% for offcuts such as head, bones, and frame, which would add 3.4 kg fish consumption per capita per year. An alternative calculation, based on increasing the current consumption of 5 kg fish ind-1 y-1 by 10 kg, would meet this target for about 18 million people, i.e. 30% of the present population. Based on current market prices, the annual gross income corresponding to the carrying capacity estimated with AQRATE would be about 270 million USD.

The carrying capacity for all of Africa, determined by scaling the freshwater and marine case studies used in AQRATE, is shown in Fig. 2. Overall, the projection for sustainable aquaculture production in Africa is between 8.8 and 10.2 million tonnes per year, bearing in mind that only a subset of the freshwater area was used.

This compares well with estimates of 11 million tonnes in 2030 in the HIGH scenario calculated using the IMPACT model (Chan et al, 2021), particularly considering that the AQRATE estimate does not include land-based ponds—Egypt alone produces around 1.6 million tonnes annually (FAO, 2024), of which 86% is pond culture.

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Includes 36 X $10^6$ t seaweeds. Animal production was 95 X $10^6$ t $y^{-1}$

The definition of offshore is complex (see e.g. Buck et al, 2023); for the purpose of this work depth is used as a criterion.

# CULTIVATION OF *Tetraselmis chui* AS A VALORISATION PROCESS FOR LIQUID SIDE STREAMS FROM SEAFOOD AND MEAT PROCESSING

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## Introduction

Microalgae are highly valuable as a source of biomass for a range of applications, from food and feed to cosmetic ingredients or biofertilisers. Moreover, they have a great potential as bioremediating organisms for wastewater from different sources due to their capacity to remove inorganic nutrients, thus mitigating environmental impacts derived from discharge or depuration costs.

The valorisation of liquid side streams through microalgae cultivation is one of the activities of IMPRESS (Innovative approaches for marine and freshwater-based ingredients to develop sustainable foods and value chains), a Horizon Europe project aimed at the co-creation, development and promotion of new and improved products and processes based on low-trophic aquatic species, both wild and farmed.

## Materials and methods

Three different liquid side streams -cooking water from octopus and tuna processing and brine from serrano ham maturation- were used to culture *Tetraselmis chui*. All three side streams contained different concentrations of inorganic nitrogen (ammonium, nitrite and nitrate) and phosphate. Tuna cooking water was rich both in nitrate and ammonium, whereas octopus cooking water and brine contained ammonium and traces of nitrate and nitrite. N:P ratio varied between 2 and 108 (Table 1). Octopus and tuna cooking were previously subjected to centrifugation to remove solid particles and oil and to membrane nanofiltration to remove protein; all these fractions are valuable for valorisation. Brine was mesh-filtered to remove large particles.

Non-axenic *T. chui* was cultured in 1-l flasks in triplicate, in the following culture media: 1) control cultures were grown in seawater supplemented with commercial nutrients by Aqualgae S.L. (Goldmedium A: 2 mM $NO_3^-$, 0.2 mM $PO_4^{3-}$ and Goldmedium B: micronutrient premix; 2) liquid side stream + Goldmedium B; 3) 50% liquid side stream + 50 % seawater + Goldmedium B. Flasks were aerated and exposed to 14 h: 10 h light:dark cycles. When necessary, NaCl was added to culture medium to provide salinity levels appropriate for *T. chui*.

Cell counts were done regularly along the culture cycle. Cultures were kept for 9-11 days, until the onset of stationary phase in control flasks. At that time, the content of the flasks was centrifuged to remove cells and culture medium was analysed to determine its microbiological and physico-chemical features.

## Results

Control, seawater cultures reached highest cell densities at the end of the growth phase. Cultures kept on 50 % liquid side stream + 50 % seawater reached similar values to control cultures, except for *T. chui* cultured in tuna cooking water. On the contrary, *T. chui* kept in pure liquid side streams did not grow, and cell densities remained constant from inoculation (Table 2).

Spent culture media showed different microbiological quality. Initial liquid side streams contained mesophilic aerobic microorganisms and Enterobacteria (Table 1). After the cultivation cycle, the content of both groups of microorganisms strongly increased in the cultures with 50 % or 100 % octopus or tuna cooking water; nevertheless, culture medium consisting in 50 % or 100 % serrano ham brine showed lower bacterial counts than the initial brine (Table 2).

## Discussion

The concentrations of inorganic N and P in the liquid side streams were enough to sustain *T. chui* growth when used at a 50 % concentration. In cultures carried out in pure side streams, turbidity caused by the rapid bacterial proliferation in seafood cooking water or due to fine particles in the case of brine could decrease light availability inside the flasks.

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Table 1. Content of inorganic nitrogen and phosphate and bacterial counts in liquid side streams used for the culture of *T. chui*.

| | Tuna cooking water | Octopus cooking water | Brine from serrano ham |
|---|---|---|---|
| $NO_3^-$ (mM) | 1.95 | 0.01 | 0.02 |
| $NO_2^-$ (mM) | 0.02 | 0.00 | 0.00 |
| $NH_4^+$ (mM) | 15.29 | 9.71 | 7.53 |
| $PO_4^{3-}$ (mM) | 0.55 | 4.70 | 0.07 |
| N:P | 31.30 | 2.07 | 107.80 |
| Mesophilic aerobic microorganisms (cfu $ml^{-1}$) | 18,000 | 90,000 | 1,200,000 |
| Enterobacteria (cfu $ml^{-1}$) | 1,000 | 1,600 | 3,000 |

Table 2. *T. chui* cell density and bacterial counts in different culture media.

| Liquid side stream | *T. chui* culture medium | Cell density ($10^6$ cells $ml^{-1}$) ± s.d. | Mesophilic aerobic microorganisms (cfu $ml^{-1}$) | Enterobacteria (cfu $ml^{-1}$) |
|---|---|---|---|---|
| Tuna cooking water | Control | 4,59 ± 0,81 | <10 | <10 |
| | 50 % | 1,90 ± 0,62 | $2.8\ 10^6$ | <10 |
| | 100 % | 0,57 ± 0,20 | $1.5\ 10^6$ | <10 |
| Octopus cooking water | Control | 5,58 ± 0,29 | $1.4\ 10^3$ | <10 |
| | 50 % | 4,20 ± 0,10 | $6.95\ 10^5$ | $1.4\ 10^4$ |
| | 100 % | 0,61 ± 0,09 | $2.7\ 10^7$ | $1.5\ 10^5$ |
| Serrano ham brine | Control | 6,21 ± 0,33 | $3.4\ 10^4$ | <10 |
| | 50 % | 5,93 ± 0,08 | $1.1\ 10^4$ | <10 |
| | 100 % | 0,66 ± 0,11 | $2.0\ 10^4$ | <10 |

Bacterial growth in both types of seafood cooking water was probably due to the content of organic carbon. Organic carbon in brine was lower, and the growth of *T. chui* prevented bacterial proliferation.

In order to use these types of liquid side streams as culture medium for microalgae, a pretreatment, e.g. pasteurisation, must be applied to prevent bacterial proliferation.

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# TURNING THE TIDE: UTILIZING INVASIVE SPECIES AS SUSTAINABLE FEED FOR JUVENILE EUROPEAN SEABASS (DICENTRARCHUS LABRAX)

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## Introduction

The rapid expansion of invasive species within marine ecosystems poses significant ecological and economic threats, frequently resulting in the displacement of native biodiversity and the destabilization of coastal environments (Mancinelli et al., 2021). However, these ecological challenges also present an opportunity: invasive species may serve as valuable, yet underutilized, biological resources. In aquaculture, their use as alternative protein sources presents a promising, sustainable solution to reduce dependence on conventional feed ingredients and support circular economy.

Along the Italian coastline, several alien species have been identified, with potential for valorization, notably:

- Whole Blue Crab (Callinectes sapidus) (a in Fig.1) – A highly invasive species in Italy, whose growing biomass is difficult to valorize. Most of its population, particularly females and smaller individuals, is currently discarded or destroyed (Azzurro et al., 2024).
- Soft Tissue from Bivalve (Anadara sp., Arc clam) (b in Fig.1) – An invasive mollusk species along the Adriatic coast with limited commercial use and low market value (Morello et al., 2004).

This study investigates the potential of incorporating these invasive species into the diet of juvenile seabass (Dicentrarchus labrax), aiming to turn an environmental liability into a sustainable asset for Mediterranean aquaculture.

## Materials and Methods

This trial was conducted at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences, University of Bologna (Cesenatico, Italy). Juvenile seabass (Dicentrarchus labrax; initial average weight 5 ± 2 g) were sourced from Panittica Italia and acclimated for two weeks. Fish (n= 100/tank) were randomly stocked into nine 450 L square tanks (three replicates per dietary treatment) and reared for ~30 days. Tanks operated within a closed recirculating aquaculture system (RAS) equipped with mechanical sand filtration, ultraviolet lights, and a biofiltration as described by Parma et al. (2023). Water parameters were maintained at 24 ± 1.0 °C, 25 ± 5.0 g/L salinity and oxygen levels at 8.0 ± 1.0 mg/L using a liquid oxygen system. Fish were fed pelleted isoproteic and isolipidic diets, containing varying levels of Anadara soft tissue meal (AM) and whole blue crab meal (BCM): 0% (control), 5% AM + 5% BCM (diet A), 5% AM + 10% BCM (diet B). Feeding was performed to satiation with a 10% surplus twice daily (8:30 and 16:30) six days per week.




**Figure 1:** Specimen of *a*) *Callinectes sapidus; b*) *Anadara* sp., Arc clam.

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At the beginning and end of the experiment, all fish are anesthetized or euthanized with tricaine methanesulfonate (MS-222). Growth performance and whole-body and dietary proximate analyses parameters are calculated. Gene expression related to digestion (amyl, lip and pept), appetite (pomc a, ghrl, cart), growth (gh, igf1, oxidative stress (sod and cat) and physiological stress (hsp70 and hsp90) are analyzed to evaluate the nutritional and physiological response to the different diets. All experimental procedures were approved by the Ethical Scientific Committee for Animal Experimentation of the University of Bologna (ID 113/2020-PR), following European Directive 2010/63/EU.

Results and Discussion

Preliminary results suggest that the inclusion of blue crab meal and Anadara meal could positively influence the growth performance and feed intake of juvenile seabass. Blue crab meal, with its high protein percentage (> 40%) and abundance of essential fatty acids and minerals (Arena et al., 2024), may promote enhanced growth rates, although its high chitin content from the carapace could pose digestibility challenges if not properly managed. Anadara meal, known to be a fish attractant could increase the ingestion rate, while its high hemoglobin-derived iron content may contribute to improved oxygen transport, enzymatic activity and immune support in fish. Nevertheless, potential risks such as heavy metal accumulation in Anadara tissues highlight the need for careful sourcing and balanced formulation (Solang et al.,2021).

Conclusion

These preliminary findings support the broader concept of utilizing invasive species to reduce dependence on conventional fishmeal, fostering a more sustainable and circular aquaculture system.

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# EVALUATING A SUSTAINABLE ORGANIC DIET IN GILTHEAD SEABREAM (*Sparus aurata*): GROWTH PERFORMANCE ACROSS TWO GENETIC LINES

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**Introduction**

Organic aquaculture is among the most demanding food production sectors in Europe, as it must adhere to the core principles of health, ecology and fairness, ensuring sustainable practices that protect animal welfare, ecosystem integrity, and social equity (European Climate, Infrastructure and Environment Executive Agency). In 2020, total organic aquaculture production in the EU reached 74,000 tonnes, accounting for 6.4% of total EU aquaculture. Within this, European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) contributed 2,750 tonnes, representing just 1.5% of the total production for these two species (Estévez & Vasilaki, 2023).

The sector is expected to grow, driven by increasing consumer demand for organic seafood. However, feed-related constraints hinder expansion: certified organic feed ingredients, especially fishmeal from trimmings, are limited, and synthetic amino acids are banned. Despite promising results with organic diets, their heavy reliance on fishmeal often makes them economically unviable. In this context, animal land-based protein sources have shown encouraging outcomes across multiple species (Galkanda-Arachchige et al., 2020). Additionally, single-cell proteins (SCP) are emerging as promising candidates for integration into feed formulations, potentially improving both sustainability and cost-effectiveness (Oliva-Teles & Gonçalves, 2001; Marchi et al., 2023). This study evaluates a novel, cost-effective organic diet for gilthead seabream, compared to both a conventional commercial and a commercial organic diet. The experimental diet is designed to match the lower fishmeal inclusion typical of conventional feeds by reducing the higher fishmeal levels commonly found in organic diets. This is achieved through partial replacement with yeast extract and certified organic poultry meal, aiming to improve economic sustainability while remaining compliant with organic standards.

**Materials and Methods**

This trial will be conducted at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences, University of Bologna (Cesenatico, Italy). Gilthead seabream (S*parus aurata* ~120 g) from two genetic lines (fast-growing and standard), (n= 30/tank) will be randomly stocked into eighteen 800 L square tanks (three replicates per dietary treatment and genetic line) and reared for ~3–4 months until reaching ~320 g. Tanks will operate in a recirculating aquaculture system (RAS) with mechanical and biological filtration and UV treatment. Water parameters will be maintained at 24 ± 1 °C, 30 ± 3 g/L salinity, and 8.0 ± 1.0 mg/L $O_2$. Three isoproteic (43%) and isolipidic (17%) diets will be tested: a conventional, a commercial organic, and a novel organic diet. Fish will be fed to satiation with a 10% surplus twice daily, six days per week. All fish will be PIT-tagged and measured for weight and length at the start and end of the trial. Growth performance (SGR, FI, FCR) will be evaluated at the tank level. Feed and carcass composition will be analyzed at the beginning and end; fillet composition, texture, colour, fatty acid profile (via HPLC), and nutrient utilization will be evaluated on 10 fish per group and diet. Blood from the same fish will be collected for plasma biochemical analysis.

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**Results and Discussion**

The novel organic diet for gilthead seabream is expected to match the growth performance of the commercial organic diet while approaching the cost-effectiveness of conventional feed. With reduced fishmeal content, partly replaced by certified organic poultry meal and yeast extract, feed conversion ratio should remain optimal and specific growth rate similar across treatments. Economic sustainability is anticipated to improve by lowering dependence on costly organic fishmeal, while maintaining compliance with EU organic standards. Fillet quality (composition, texture, colour, fatty acids) should remain within acceptable organic ranges, with only minor lipid class changes from protein source differences. Plasma biochemical values are expected to stay within physiological limits, indicating no health issues. Overall, incorporating certified poultry meal and yeast extract could enable cost-effective organic diets that maintain growth, quality, and welfare, supporting the sustainability and expansion of EU organic aquaculture.

**Conclusion**

The novel organic diet, incorporating certified poultry meal and yeast extract to partly replace fishmeal, is expected to sustain growth, fillet quality, and fish health comparable to commercial organic feed, while lowering costs and promoting sustainable seabream production.

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# TRANSFER OF MICROPLASTICS BY PACIFIC OYSTER INTO THE FOOD CHAIN: IMPACT IN SHELLFISH AQUACULTURE

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**Introduction**

Microplastic pollution in marine ecosystems has become a global issue with adverse effects on biodiversity and aquaculture. They pose a physical threat to marine organisms and can act as vectors for contaminants, amplifying their impact on the food chain. Oysters are filter-feeding organism, thus particularly vulnerable to microplastics (MPs) and associated contaminants, resulting in a food safety risk.

This study aims to elucidate whether the Pacific oyster (*Magallana gigas*), one of the most important species in mollusc aquaculture worldwide,) act as a vector of microplastics transfer to the food chain (and what would be the role of the presence of hydrocarbons adsorbed on them in this transfer) and ii) what would be their behavior in the event of a possible oil spill in the water.

**Material and methods**

Individuals of *Magallana gigas* (approximately 10,06 - 13,22 cm from hinge to shell margin) were obtained from the Jose Mº Daporta Leiro e Hijos S.L. enterprise, located in Ria de Arousa, Galicia, Spain. They were maintained in 4L glass aquaria with natural seawater (temperature: 17-20ºC and salinity: 37-37.5 ppt), under constant aeration and a water renewal rate of 1L day-1. They were fed daily with *ReefPulse* from Ocean Nutrition. Oysters were acclimated to the tanks and laboratory conditions for 7 days before being used in experiments.

Contaminated and uncontaminated MPs were administered to each oyster with tweezers, and their percentage of ingestion and retention time were evaluated. The microplastics used were green, pink, and black Gütermann polyester fibres, 2 mm in length. Contamination with diesel was performed as follows. MPs were mixed with 50 ml of 100% diesel at 150 rpm for 72 hours and then washed with seawater at 170 rpm for 28 hours to remove excess non-adsorbed diesel. Uncontaminated MPs were prepared following the same procedure using seawater instead of the diesel solution.

Individual oysters were submersed in a diesel-seawater mixture (ratio 1:1000) for 24 hours. Their filtering activity (time with their valves open) was evaluated by observing them for 10-minute cycles during 5 days (including the 24-hour exposition).

**Results and discussion**

Oysters ingested 92% of the polyester fibres supplied, with an average of around 4,9 particles per individual and 0.2 particles per gram of wet tissue, without showing a statistically significant colour influence. The presence of hydrocarbons did not affect their palatability, but microplastics with adsorbed diesel were retained significantly longer (6 h of average) before being expelled, with a maximum retention of 24h, while uncontaminated ones had a mean of 21 min. Additionally, an increased filtration activity was observed in oysters exposed to diesel, raising questions about the physiological impact of contaminants on oysters as well as their ability to adapt to polluted environments.

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**Conclusions**

Aquaculture is a key activity to food supply and economic development, thus understanding the interaction between farmed organisms and emerging contaminants is essential for designing mitigation strategies and ensuring the viability of this activity in future. This study provides relevant information on the interaction between oysters, microplastics, and diesel pollution, with implications for aquaculture. According to the results, oysters can be considered a vector for transferring microplastics into the food chain. Furthermore, the time window in which microplastics would be available for transfer to a predator depends on the substances absorbed in them, highlighting the ability of microplastics to act as carriers of other contaminants. It has also been shown that oysters are sensitive to the presence of diesel in the water, changing their behavior significantly (p-value = 0,0000364). Further experiments should be conducted to determine what implications this might have for their commercialization for human consumption.

**Acknowledgements:** This study forms part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGeneration EU (PRTR-C17.I1) and by Generalitat Valenciana (GVA-THINKINAZUL/2021/027; Principal Investigators: C. Amaro and B. Fouz, Universitat de València (UV) and GVA-THINKINAZUL/2021/043; Principal Investigators: C. Bordehore and D. García, Universidad de Alicante (UA)).

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# THE DELICATE BALANCE BETWEEN IRON DEFICENCY AND EXCESS IN AQUATIC SPECIES: A SHORT REVIEW

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*Introduction: Essentiality of Iron (Fe)*

By playing an essential role in the activation and modulation of several processes involved in immune response, optimal trace mineral nutrition is central in helping fight stress and disease in the increasingly challenging production conditions of farmed aquatic animals. Iron is a trace mineral of fundamental importance to the health and performance of farmed aquatic species. It is required for several biochemical processes, including electron transfer reactions, gene regulation, the binding and transport of oxygen, and regulation of cell growth and differentiation. Our presentation aims to explore the essential roles of Fe in fish, focusing on its impact on hemoglobin synthesis and mitigation of anemia, immune cell functionality, stress resilience, and the delicate balance between deficiency and excess. Recommendations on dietary Fe supplementation strategies will be drawn from this short review of published literature.

*Primary Signs and Causes of Fe Deficiency*

Iron deficiency can impair immune response, making fish more susceptible to infection and environmental stressors. Deficiency primarily manifests as hypochromic microcytic anemia, which is characterized by reduced hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin. This condition leads to decreased growth rates, reduced appetite, lower feed efficiency, and poor overall performance. Characteristic hypochromic microcytic anemia has been detected in various fish species, including brook trout, rainbow trout, Atlantic salmon, red sea bream, yellowtail, eel, and carp fed low-Fe diets. In catfish, Fe deficiency suppressed hematocrit, hemoglobin, and plasma Fe levels, and caused transferrin saturation. Although gills play an important role in Fe acquisition, particularly in developing fish where the digestive tract is not fully functional, the gastrointestinal tract is considered the major site of Fe absorption. Replacement of fish meal with alternative ingredients lower in Fe content and bioavailability requires proper Fe balancing to avoid nutritional deficiency and the associated negative impacts on performance and health of fish.

*Non-Nutritional Causes of Anemia*

Anemia in fish can also be caused by infection and infestation. Viral and bacterial infections, such as infectious salmon anemia virus (ISAV) and *Aeromonas hydrophila,* can cause anemia. Parasites, such as *Argulus sp.* and *Trypanosoma sp.* contribute to anemia through blood loss and hemoglobin digestion. Parasitic infections are a growing concern in mariculture. More specifically, monogeneans are among the most threatening ectoparasites in aquaculture due to their impact on economically relevant finfish species, including gilthead seabream. Sparicotylosis, caused by *Sparicotyle chrysophrii*, leads to severe anemia and histopathological lesions in the gills, and represents the number one health concern in gilthead seabream production units under offshore farming conditions across the Mediterranean Sea. We will explore how optimization of Fe supplementation may help mitigate the impact of these infections and infestations, which seem to have become more common and severe with current observed climate changes.

*Current Iron Requirements of Fish*

The Fe requirements of fish vary, depending on species, age, and physiological conditions. Revised by Lall and Kaushik (2021), and Prabhu *et al*., (2004), the minimal dietary inclusion level of Fe is influenced by factors such as the type of experimental diet used, dietary interactions, and the mineral concentration of water. Hemoglobin levels are considered appropriate criteria for determining Fe requirements. For example, Atlantic salmon requires Fe levels that support hemoglobin regeneration and overall health, with bioavailability varying significantly among different Fe sources. Research indicates that ferrous sulfate and ferric chloride have similar bioavailability, while Fe from blood meal and Fe-hydroxy methionine analogues show higher bioavailability. In Nile tilapia, Fe-amino acid complex showed to be a better source than inorganic Fe to improve performance, enhance hemoglobin, and reduce oxidation. Specific dietary Fe requirements for various fish species are generally in the range of 30 to 100 mg/kg of diet.

*(Continued on next page)*

*The Delicate Balance Between Fe Deficiency and Excess*

Maintaining a balance between Fe deficiency and excess is essential for optimal fish health. While Fe deficiency can impair hemoglobin synthesis and immune function, too much Fe or provided in certain forms, can promote oxidation of feed components (e.g. vitamins, fatty acids and astaxanthin), affecting fish performance, health and organoleptic properties. Ingredients such as blood meal have a high content of haem-Fe, and although of relatively good bioavailability, it may also promote oxidation of feed components. Excess Fe in chemical forms that become readily available in the gut can also promote bacterial growth and oxidative stress. Iron not efficiently absorbed may feed pathogenic bacteria, which ultimately affects fish health. A decrease in the amount of Fe available to the human gut microbiome reduced the growth of potentially pathogenic bacteria. Growth of *Escherichia coli and Salmonella typhimurium* were significantly impaired when cultured independently in Fe-deficient media. In contrast, depletion of Fe did not affect the growth of the beneficial species, *Lactobacillus rhamnosus*, when cultured independently. In Atlantic salmon, Fe overload was shown to modulate the immune response in fish and increase susceptibility to *Piscirickettsia salmonis* infection. Moreover, Fe chelated with deferoxamine (DFOA), a pharmaceutical compound used to bind Fe and aluminium, enhanced salmon survival and reduced bacterial load. This indicates that chelated Fe sources, in comparison with inorganic and elemental forms of Fe, are better at positively modulating the gut microbiome of fish. Replacement of an inorganic trace mineral premix with metal-complexed minerals showed a positive modulation of the microbiome in tilapia intestine, which may be at least partly related to the replacement of an inorganic source Fe source with a form of complexed Fe included in the premix.

Iron is a fundamental trace mineral for fish health, playing crucial roles in oxidation-reduction activity, oxygen transport, and immune function. Maintaining a balance between dietary Fe deficiency and excess is essential for optimal fish health and performance. This presentation highlights the importance of optimizing Fe supplementation levels and sources to ensure the health and productivity of farmed aquatic species.

# CHROMIUM-METHIONINE COMPLEX IMPROVES PERFORMANCE AND RETENTION OF PROTEIN AND PHOSPHORUS IN GILTHEAD SEABREAM

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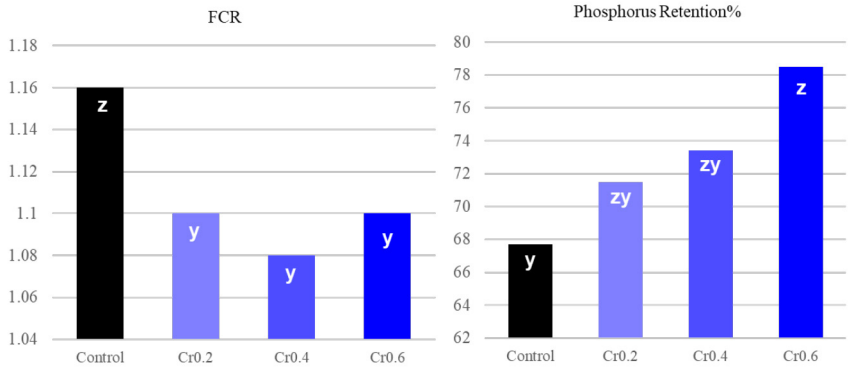
Although carbohydrates are the cheapest dietary energy source and essential for producing high-quality pellets, elevated carbohydrate (glucose) levels can pose nutritional challenges for fish compared to land animals. Dietary supplementation with Cr methionine (Cr-Met) complex proved to be consistently effective in improving utilization of glucose and feed utilization in fish, regardless of their trophic level (from carnivorous to omnivorous species), optimum temperature (warm- to cold-water species) or salinity tolerance (seawater to freshwater). The efficacy of graded dietary levels of Cr-Met complex, in the form of Availa®Cr 1000, was recently evaluated in terms of performance and whole-body nutrient retention in gilthead seabream (*Sparus aurata*). The trial was comprised of four dietary treatments: a control diet (Control) without Cr-Met complex supplementation; and three other diets, based on the Control formulation, but supplemented with Cr-Met complex at increasing doses (0.2, 0.4 and 0.6 mg.kg$^{-1}$). Diets were isonitrogenous (crude protein, 41.8%), isolipidic (16.3%) and isoenergetic (gross energy, 21.6 MJ/kg). Quadruplicate groups of 50 gilthead seabream, with a mean initial body weight of 32.1 ± 2.1 g were fed one of the four experimental diets for 91 days. At the end of the trial, the final body weight ranged between 126.4 and 134.7 g. When compared to Control, supplementation with Cr-Met complex resulted in significant improvements for specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER); being highest at 0.4 mg.kg$^{-1}$. The positive effect of Cr-Met complex on seabream performance was associated with a better utilization of glucose, as indicated by the significantly lower activities of PEPCK and significantly higher activities of HK and GK in the liver of fish fed diets supplemented with the highest Cr-Met complex levels. By improving the utilization of glucose (dietary carbohydrates), Cr-Met complex spared the utilization of protein for growth purposes which turn out in 15% more protein being retained in the body. Interestingly, supplementation with Cr-Met complex has also significantly improved phosphorus retention; 16% more P retention at 0.6 mg.kg$^{-1}$. In summary, improvement of carbohydrate utilization with the supplementation of Cr-Met complex is proven to be an effective strategy to improve seabream growth, feed efficiency, N and P retention and consequently minimize nutrient excretion in aquaculture. Harnessing a unique mode of action, it represents a complementary tool to support the implementation of improved feeding strategies aiming to advance sustainability goals and profitability of aquaculture operations.



# EFFECT OF SMOLTIFICATION REGIMES ON VACCINE EFFICACY; DEMONSTRATED IN A CLINICAL STUDY WHERE ALPHA JECT MICRO 7 ILA VACCINATED A. SALMON HAVE BEEN CHALLENGED WITH ISAV

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Over the last three decades Infectious Salmon Anaemia (ISA) has periodically caused high mortality in Atlantic salmon (*Salmo salar*) aquaculture. The severity of the outbreaks in terms of mortality levels and virus detection (both virulent and low /non-virulent) has varied both within and between sites and has primarily been handled by stamping out, removing affected cages, or intensified surveillance.

PHARMAQ developed an ISA challenge model in 2002, and later demonstrated proof of concept for an ISA vaccine based on a European subtype of the virus. Vaccine efficacy was further demonstrated in evaluated challenge models (both IP and cohabitation), which contributed to the documentation of multi- (ALPHA JECT® 5-1 and ALPHA JECT ® micro 7 ISA) vaccine for salmonid markets.

The last decades there have also been an intensification of smolt production in all markets mainly caused by larger hatcheries, often with RAS technology. The combination of technologies result in off-season smolt production based on different smoltification protocols where different light regimes and smoltification feed, are among the most used. There are concerns related to suboptimal smoltification creating stress and further suppressed immunity (both specific and unspecific) and vaccine response. The current study evaluates different smoltification protocols followed by vaccination with ALPHA JECT ® micro 7 ILA and by ISAV challenge (both IP and cohabitation). The vaccine efficacy against experimental challenge and applicability combined with experiences from commercial use of our ISA vaccines will be presented, together with discussions related to best practice smoltification protocols combined with timing of vaccination.

The use of experimental animals was approved by the Norwegian Food and Safety Authority and internal ZOETIS Animal Ethics Committee.

# PREVALENCE AND RESISTANCE POTENTIAL OF *Flavobacterium columnare* AND *Flavobacterium psychrophilum* FROM AQUACULTURE IN BELGIUM

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This study aims to understand the distribution of *Flavobacterium columnare* and *Flavobacterium psychrophilum* as pathogens in aquaculture farms and to assess their resistance to antibiotics. These are significant bacterial pathogens in freshwater aquaculture, responsible for columnaris disease and cold-water disease (CWD). These pathogens can cause substantial losses in farmed fish populations, particularly under stress and poor environmental conditions. As part of the European Partnership on Animal Health and Walfare (EUPAHW) project, winter sampling campaign was conducted at aquaculture farms across the regions of Belgium, involving nine companies: five trout companies in Wallonia and four companies of sturgeon, mussel, oyster, trout, bulleye, burbot, pikepearch in Flanders. Samples collected included skin and gill mucus, water, and sediment. From these, 86 presumptive isolates of *F. psychrophilum* were recovered using selective media tryptone yeast extract salts agar (TYESA) with tobramycin and neomycin and 39 of *F. columnare* were recovered using modified shieh agar with tobramycin and preliminary phenotypic identification. To ensure accurate species confirmation, all isolates will undergo identification using Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDITOF MS) and specific quantitative Polymerase Chain Reaction (qPCR). We are currently identifying of the bacterial isolates with MALDITOF and qPCR. We will look into antimicrobial resistance (AMR) of the two pathogens after confirmation of their exact identity by performing minimum inhibitory concentration (MIC) test.

# AUTOMATED DETECTION OF BARBED WIRE JELLYFISH IN CITIZEN SCIENCE IMAGERY

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## Introduction

Barbed wire jellyfish (*Apolemia sp.*) is a marine organism that self-organizes into string-like colonies of length ranging from a few centimetres to over 30 metres. The individuals that make up a colony contain hair-like structures with stinging cells – making a colony resemble barbed wire both in look and touch. The genus is distributed in the Mediterranean, Pacific, and Atlantic seas. In recent years, it has been observed with great abundance along the Norwegian coast, both intact colonies and swarms of fragments. Such swarms have caused significant disruption of aquaculture operations and mass mortality events in salmon fish farms, resulting in economic losses.

The new appearance of barbed wire jellyfish along the Norwegian coast, and the ensuing challenges for Norwegian aquaculture, necessitate a better understanding of their distribution and abundance. However, these gelatinous zooplankton (such as Apolemia sp.) are often overlooked in Norwegian marine monitoring programs. Moreover, traditional sampling techniques are poorly suited for capturing gelatinous organisms, leading to significant knowledge gaps regarding their distribution, abundance, and seasonal dynamics across Norwegian waters. To address these challenges, the Norwegian citizen science portal "Dugnad for Havet" (DfH) [1] was launched in 2019.

Using expert-validated images from DfH, we have developed and benchmarked machine learning models which detect barbed wire jellyfish in citizen science images. We describe these models and their performance below. The best-performing model is intended for integration into DfH to automate identification of Apolemia sp. and is part of the development of an early-warning system through the JellySafe project.

*Acknowledgements:* We are grateful to Sajidah Ahmed, Marius Andersen, and Øystein Knutsen for discussions and assistance with various activities related to this text, including data cleaning, labelling, and model development. This activity was funded by the FHF through the project JellySafe (FHF/ 901941).

## Methodology

*The «Dugnad for Havet» (DfH) dataset.* The DfH project [1] by the Norwegian Institute for Marine Research invites participants to submit observations of gelatinous zooplankton. Each observation includes fields such as photograph(s) and/or video (optional), proposed species identification, geographical coordinates, date and time. Observations have been submitted by a broad range of users, including recreational divers, fishermen, as well as aquaculture personnel. Most images were captured using smartphones from the sea surface.

Between January 2019 and April 2025, there were 4080 observations of gel. zooplankton recorded. Notably, the siphonophore Apolemia sp. accounted for over 50% of the total observations, with 2068 records and approximately 1400 accompanying images collected to date. To ensure data reliability, each submitted record undergoes a manual validation process conducted by gelatinous zooplankton specialists.

Despite its many advantages, the DfH citizen science approach presents several inherent biases and challenges, including sampling bias, seasonal bias, observer bias, and a high validation workload. By developing models which automate identification, we aim to improve the efficiency of the validation process and leverage the growing volume of submitted images, focusing first on the most reported and harmful species Apolemia sp.

*Machine learning: architectures and training.* Citizen science images present challenges for object detection such as variable lighting, varying resolutions, camera settings, etc. After strict data cleaning, our Apolemia sp. dataset consisted of 350 images. To deal with small datasets, leveraging pre-trained models is a common strategy. We compare the recent state-of-the-art models YOLOv12 [2] and RF-DETR [3] on our detection task.

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The YOLO family, originally based on Convolutional Neural Networks (CNNs), has long dominated one-stage detection due to its efficacy and speed. While early versions primarily relied on local receptive fields, recent models (e.g., YOLOv12) increasingly incorporate attention mechanisms to capture broader context. In parallel, transformer-based detectors such as DETR and its successors (e.g., RF-DETR) fully embrace global attention, offering advantages in cluttered or complex scenes. These architectures may therefore excel in detecting complex or cluttered scenes, such as the elongated and diffuse morphologies of barbed wire jellyfish.

Both models were trained with 10-fold cross-validation and standard augmentations[1].

**Results and discussion**

The models were evaluated on their mean Average Precision (mAP) [4] scores, which is the percentage of objects correctly detected. Here, 'detect' means that the model predicts a bounding box covering a threshold of the ground truth bounding box. We use a threshold of 50% to indicate detection skill, and average across a threshold range of 50%-95% to reward more precise outlines.

Both the models YOLOv12 and RF-DETR performed well on the detection task, achieving mAP 50% scores of 94.0% and 91.4%, respectively. Their mAP 50-95% scores were essentially identical, at respectively 76.4% and 76.3%. This indicates that the YOLOv12 model was able to correctly detect more of the Apolemia sp. targets, but that the RF-DETR bounding boxes were slightly more precise.

*Future directions.* Our models are intended to reduce the validation workload of DfH and to be part of an early-warning system for harmful jellyfish blooms along the Norwegian coast. This development is ongoing through the JellySafe project [ref]. As more images become available through DfH, we also aim to include other gelatinous zooplankton.

A drawback of the bounding box models presented above is that they don't give precise quantity estimates. To address this, we are investigating pixel-wise segmentation models.

A standard mosaic dataloader was only available for YOLOv12, not RF-DETR.

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# CONSUMER HEALTH BELIEFS AND ACCEPTANCE OF SALMON FED WITH CHICKEN INGREDIENTS: APPLICATION OF THE HEALTH BELIEF MODEL

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**Introduction:**
The salmon industry is seeking alternative protein sources to include in feed formulations. Fishmeal and soy protein, currently the primary protein sources in salmon feed, are criticized for their environmental impacts. However, finding an alternative protein source that is available in high volumes, cost-competitive, nutritionally adequate, and environmentally sustainable remains challenging. In this context, processed animal protein from chicken could represent a promising alternative. Nonetheless, consumer acceptance of integrating chicken into salmon feed remains a concern. European consumers may perceive feeding salmon with land-animal ingredients as unhealthy or unnatural. This study investigates how consumers' health beliefs about salmon fed with chicken ingredients influence their acceptance of such products. The Health Belief Model (Rosenstock, 1974) is the theoretical framework to explore these relationships (Figure 1).

**Material and methods.**
The study is based on survey data of 500 French and English consumers. Confirmatory factor analysis is used to assess the validity of the latent variables (value, belief, norm, empathy and intention). A structural equation modelling analysis with latent constructs is used to test the hypotheses.

**Results**:
We expect to validate the ability of the Health Belief model to explain consumer acceptance of salmon fed with chicken and uncover consumers' health beliefs regarding salmon fed with chicken and their effect on behaviour.

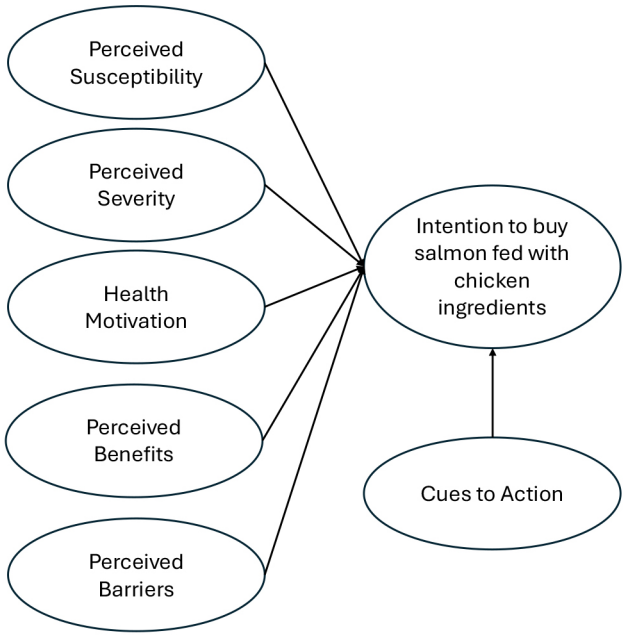


Figure 1. Health Belief Model (Rosenstock, 1974).

**Reference:**
Rosenstock, I. M. (1974). Historical origins of the health belief model. *Health education monographs*, *2*(4), 328-335.

# DEVELOPMENT OF ANIMAL HEALTH MODELS FOR EMERGING PATHOGENS IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) IN THE MEDITERRANEAN

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## Introduction

Bacterial diseases represent a significant issue in Mediterranean aquaculture. The number of new pathogens identified has increased over time, and one of the most common causes of the emergence of infectious diseases in marine fish is the *Aeromonas* species (Tansel Tanrikul & Dinçtürk, 2021). Over the past decade, *Aeromona veronii* has become a major problem in European sea bass (*Dicentrarchus labrax*) farming in some Mediterranean countries, with outbreaks occurring when water temperatures reach their highest values (Smyrli et al., 2019). Due to factors such as climate change, the duration of periods with higher water temperatures is increasing, which not only leads to a higher incidence of outbreaks but also allows them to persist over time. Additionally, the rise in water temperatures contributes to the proliferation of other emerging pathogens (Combe et al., 2023). Due to current restrictions on the use of chemicals or antibiotics in aquaculture production, effective and sustainable preventive treatments against systemic infections in cultured species are required (Yilmaz et al., 2022). To assess their effectiveness, the development of new standardized fish health models is necessary. In response to this challenge, we are working on the development of infection models for *A. veronii*, with plans to expand them to other emerging pathogens such as *Lactococcus garvieae*. To date, we have standardized two infection models for *A. veronii*, using intraperitoneal (IP) injection and bath immersion. These models have been tested under different conditions to evaluate their reproducibility. Preliminary results suggest that pathogenicity varies significantly with temperature, but it is also influenced by factors such as fish size, fish batch, and culture density, among others. Our next steps involve developing and validating a cohabitation infection model for *A. veronii*, as well as extending this approach to other emerging pathogens.

## Materials and methods

Bacterial isolates from internal organs of a sick fish (real outbreak in a Turkish farm) were streaked on tryptic soy agar (TSA) plates supplemented with 2% NaCl. The TSA plates were incubated at 25°C for 24-48 h and colony morphology was observed. The pure colonies were streaked again on TSA plates to determinate the specie by proteomic analyses (MALDI-TOF). Once confirmed, the *A. veronii* strain was cultured for cryopreservation and added to the CTAQUA strain collection (CT0068). A stock of different vials was generated from a liquid culture in tryptic soy broth (TSB) supplemented with 2% NaCl, with bacteria grown for 24 hours at 25 °C under agitation (150 rpm). Subsequently, bacteria were centrifuged to remove the culture medium and resuspended in a cryopreservation medium (50% glycerol in Phosphate Buffer Saline (PBS)) at a final concentration of $1\times10^9$ Colony Forming Units (CFU)/mL. Vials were stored at -80 °C. After an initial virulence test in which 10 European sea bass were inoculated by IP injection to confirm that the strain caused mortality, dose-response trials were conducted. Infection by IP injection was carried out by inoculating 0.1 ml of bacterial suspension per fish. This suspension was prepared by diluting a cryovial with an initial concentration of $1\times10^9$ CFU/mL in PBS to obtain the concentrations to be tested. Bath infection was carried out by scaling up the culture in TSB supplemented with 2% NaCl at 25°C under agitation (150 rpm) from one of the cryovials (1 mL) until obtaining a sufficient amount of culture at a concentration of $1\times10^9$ CFU/mL to prepare the dilutions to be tested in 10-liter buckets with seawater. Then, fish (20 gr) were placed in the buckets for 1 hour with aeration. The handling of the animals was performed under deep anesthesia induced by an anesthetic (benzocaine). In all the tests conducted, a control group was included, in which the fish were handled in the same way as the experimental fish but without exposure to the pathogen. Control fish were inoculated with PBS in the case of IP injection or placed only in seawater in the bucket for bath immersion. All concentrations were validated in triplicate (10 fish/tank) and after pathogen exposure, the fish were transferred to tanks in a Recirculating Aquaculture System (RAS), where all culture parameters were monitored throughout the trial. IP injection tests were carried out at 22°C, while the bath tests were conducted at 22°C and 25°C to evaluate the dose-response at different temperatures.

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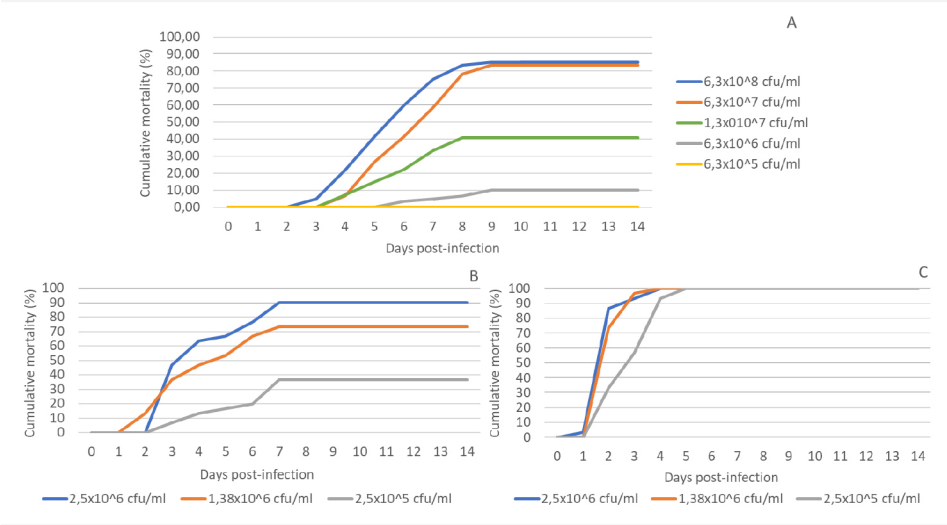


Graph 1. Mortality curves for: A) IP injection at 22 °C, B) bath at 22 °C and C) bath at 25°C.

## Results

Graph 1 shows the cumulative mortality results for the different bacterial concentrations of *A. veronii* according to the tested route of administration and temperature.

In sight of the results, it can be concluded that there is a good dose-response relationship for the CT0068 isolate when administered to pre-ongrowing sea bass both by IP injection and by bath, making these models suitable for validating preventive strategies and treatments against *A. veronii*.

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# MORPHOLOGICAL EFFECTS OF NOVEL FEED COMPOSITIONS ON INTESTINE AND SPLEEN OF GILTHEAD SEABREAM (*Sparus aurata*) BROODSTOCK

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## Introduction

The sustainability and environmental effect of conventional feed ingredients is a growing concern in aquaculture. In order to increase aquaculture sustainability, alternative protein sources are being studied. In a previous study, conducted on the same gilthead seabream (*Sparus aurata*) broodstock involved in the present analysis, no stress responses were detected following six months of feeding with diets incorporating insect and algal meals. To further characterize the effects of these alternative protein sources, we evaluated intestinal and splenic histological parameters in the same experimental population.

## Materials and Methods

In this study, three experimental diets were investigated: a control diet (CTRL) composed with conventional protein sources, and two novel diets containing a mix of four alternative protein meals, in detail *Hermetia illucens larvae, Lemna minor, Nannochloropsis gaditana*, and *Alaria esculenta* at 5% (L1) or 10% (H1) inclusion levels. For six months Gilthead seabream broodstock (n=66, equally divided in three groups) were fed with the three experimental diets. Sample from intestine and spleen were taken, fixed in formalin and embedded in paraffin. Using a microtome, tissue sections of 4 μm in thickness were produced, and they were then stained with hematoxylin and eosin. Goblet cells in the intestinal mucosa were counted, and melanomacrophage center (MMC) regions in the splenic parenchyma were measured as indicators of mucosal defense and chronic stress response, respectively. Statistical analyses were conducted using a linear mixed model; significance was set at $p<0.05$.

## Results

A significant difference was found in goblet cell counts (Fig. 1) among diets ($p<0.05$), with higher counts in the control group. However, inflammatory signs, particularly hyperplasia, were sporadically noted in fish fed L1 and H1 diets. The MMCs (Fig. 2) percentage coverage in the spleen did not show any significant variation among dietary treatments.

## Discussion

The lower number of goblet cells in fish fed the L1 and H1 diets suggests that these formulations did not cause a mucosal defense response in the intestine. However, the presence of intestinal inflammation indicates that alternative protein sources—despite being associated with no significant systemic stress responses and lower goblet cell counts—may still impact gut health in gilthead seabream broodstock. Splenic histology, like the measured stress biomarkers, appeared unaffected.

## Conclusion

Although the different diets did not induce oxidative or stress-related responses, the observed intestinal histological alterations suggest that further investigation into gut inflammatory responses is warranted to ensure these dietary formulations do not compromise the performance and welfare of gilthead seabream.

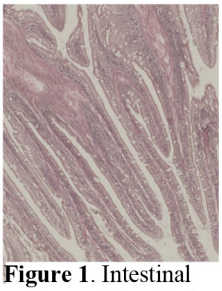

**Figure 1**. Intestinal mucosa with goblet cells

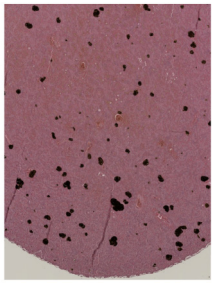

**Figure 2**. Melanomacrophage center in spleen

# DIET-INDUCED MODULATION OF THE INTESTINAL MICROBIOTA IN *Seriola dumerili*: A COMPARATIVE ANALYSIS OF FISHMEAL, PLANT-BASED, AND ALGAL-SUPPLEMENTED DIETS

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**Introduction**

The greater amberjack (Seriola dumerili) is a promising candidate for diversifying marine aquaculture because of its rapid growth and consumer acceptance. However, it requires high levels of protein, mainly from fishmeal. Therefore, developing plant-based diets that support good growth rates and encourage sustainable sector development is essential. The lower digestibility and availability of plant ingredients can negatively impact fish health. To address this, strategies are needed to improve the absorption and digestibility of plant-rich diets. One such approach involves enhancing the uptake of diets high in plant ingredients through biotechnological pre-treatments, such as enzymatic hydrolysis and/or supplementing with nutraceuticals derived from algae. We assessed the impact of partial fishmeal replacement with plant proteins (PP) and an algal-supplemented plant-based diet (PPLB) on the composition and function of intestinal microbiota.

**Materials and Methods**

Fish were reared in 400 L tanks at 22°C and 37‰ salinity, fed ad libitum for 69 days. Three isoproteic and isolipidic diets were tested: FM (fish meal), PP (50% fishmeal replacement by plant protein with biotechnological pre-treatment), and PPLB (PP + 3% algal nutraceutical). Anterior intestinal sections (mucosa plus digesta) from six fish per group were preserved in DNA/RNA Shield. Microbiota was analysed via 16S rRNA sequencing (Illumina NextSeq, 600 cycles, P1 Kit, V3–V4 region, Zymo Research). DADA2 and QIIME were used for denoising and taxonomic classification. LEfSe identified diet-associated microbial biomarkers.

**Results**

Phylum-level composition showed dominance of *Proteobacteria* and *Firmicutes* across all diets, with *Actinobacteria* enriched in PPLB. LEfSe identified 31 discriminant taxa: PPLB (17), FM (8), and PP (6). PPLB diets enriched *Propionibacterium*, *Rothia*, *Dechloromonas*, *Carnobacterium*, *Marinobacter*, and *Nocardioides*, which are taxa linked to fermentation (associated with SCFA production), immunomodulation, and adaptation to algal substrates (Nakamura *et al*., 2020; Xiao *et al*., 2022, Pinto *et al*., 2021). FM-associated taxa included *Veillonellaceae* and *Negativicutes*, suggesting a core anaerobic commensal community (Facchin *et al*., 2024).

PP enriched mainly environmental taxa (e.g., *Paraburkholderia*), with limited functional specialisation. This limited microbial adaptation may reflect decreased digestibility or absence of bioactive compounds in non-supplemented plant-based diets.

The cladogram visualising phylogenetic relationships among the identified biomarkers supported the compositional findings (Fig. 1). Taxa enriched in PPLB clustered into well-defined clades, particularly within *Actinobacteria* and *Proteobacteria*, indicating coordinated shifts in microbial lineages in response to dietary supplementation. PP-associated biomarkers appeared more phylogenetically dispersed, suggesting less targeted adaptation of the microbiota. FM-associated taxa clustered into anaerobic and mucosa-adapted branches, consistent with their ecological roles.

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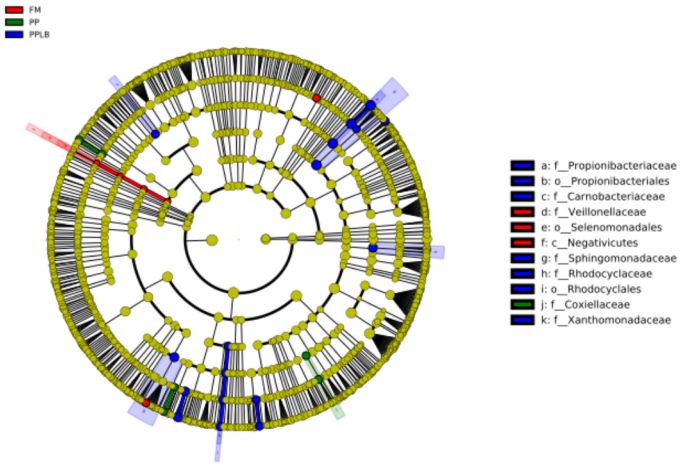


**Figure 1.** Phylogenetic cladogram of bacterial taxa differentially abundant across diets. Taxa are colour-coded by diet and enrichment type and arranged according to their taxonomic hierarchy. Converging clades highlight coordinated lineage-level shifts, particularly in the PPLB group.

Conclusion

The algal-supplemented PPLB diet promoted a diverse, functionally enriched intestinal microbiota in *S. dumerili*, whereas the PP diet alone induced limited adaptation. These findings support the use of biotechnologically enhanced algal nutraceuticals to improve gut health in sustainable aquafeeds.

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**Acknowledgments**

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# EFFECTS OF KRILL MEAL ON HYPOXIA TOLERANCE IN RAINBOW TROUT (*Oncorhynchus mykiss*): EXPLORING SEX-DEPENDENT RESPONSES

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## Introduction

Salmonids are sensitive to low dissolved oxygen (DO) levels, with sudden drops often triggered by high temperatures, fish density, or handling. Climate change may increase the frequency of such events, affecting fish welfare and performance. Antioxidant micronutrients like selenium, vitamin C, and astaxanthin are promising tools to enhance stress resilience. Selenium supplementation has been shown to improve hypoxia tolerance in trout fry fed plant-based diets (Wischhusen et al., 2020), with evidence suggesting sex-related differences in oxidative stress responses (Schomburg and Schweizer, 2009). Krill meal (KM), naturally rich in antioxidants, has shown benefits for growth, flesh quality, and fish health (Kaur et al., 2022, Tran et al., 2025). This study investigated the effects of KM on juvenile rainbow trout performance, antioxidant responses under hypoxia, and sex-related metabolic differences.

## Materials and methods

Four experimental diets were tested: a control diet (CTRL) containing 10% fishmeal, and three diets in which krill meal (KM) replaced the marine fraction (fishmeal + fish oil) at 5% (KM5), 7.5% (KM7), and 10% (KM10). Groups of 60 juvenile rainbow trout (37 ± 1 g), of mixed sex, were randomly distributed into triplicate tanks per treatment and fed their respective diets for 12 weeks at 17 ± 1 °C, with DO maintained above 9 mg/L. At the end of the feeding period, 30 fish per tank were euthanized for tissue collection. The remaining fish were subjected to chronic hypoxia (< 6 mg/L DO) for an additional 3 weeks before final sampling. Growth performance, feed intake, and survival were monitored throughout the trial. Feces were collected by stripping for digestibility analysis. Fillet color, sex, whole-body composition, liver antioxidant status, plasma stress indicators, and blood proteomic profiles were assessed. Data were analyzed by ANOVA and presented as means ± SEM.

## Results

Fish fed the KM7 diet showed improved growth performance both before and after the chronic hypoxia challenge (Fig. 1A). Chronic hypoxia significantly reduced fillet redness, whereas KM inclusion enhanced it in a dose-dependent manner, likely due to its natural astaxanthin content, as all KM groups showed higher redness than the control both before and after hypoxia (Fig. 1B).

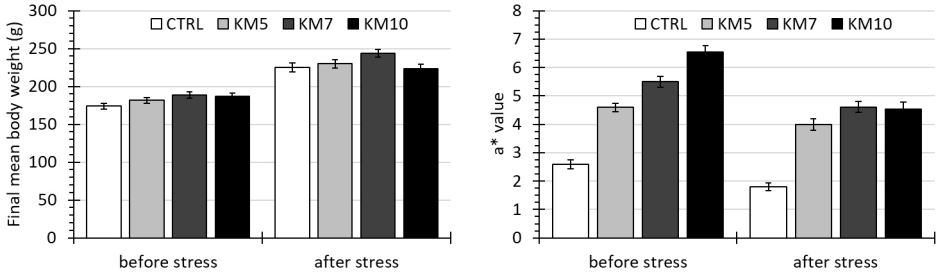


Fig. 1. Growth performance (A) and fillet color with the intensity in red (B) in juvenile rainbow trout fed the experimental diets before and after hypoxia

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**Discussion**

This study shows that KM inclusion improves growth performance and fillet quality in juvenile rainbow trout, particularly under chronic hypoxia. The 7.5% KM diet (KM7) was especially effective, suggesting that antioxidant-rich ingredients such as KM can enhance fish resilience to oxygen deprivation. Improved fillet redness under hypoxic conditions, likely linked to dietary astaxanthin, further supports this protective effect. These findings are consistent with previous work by Kalinowski et al. (2019), who demonstrated that dietary astaxanthin improved hepatic oxidative stress tolerance in trout exposed to hyperoxia. The antioxidant components of KM, including astaxanthin and selenium, may thus play a key role in mitigating stress-induced oxidative damage.

A notable finding is the potential for sex-dependent differences in hypoxia responses. Ongoing analyses aim to determine whether the effects of KM differ between males and females, particularly regarding antioxidant pathways. Previous studies have reported sex-specific oxidative stress responses (Schomburg and Schweizer, 2009), but these remain to be fully characterized in the present study. Further investigation of liver antioxidant status, plasma stress markers, and whole-body composition will provide deeper insights into the physiological adjustments associated with hypoxia and KM supplementation.

**Conclusion**

KM appears to improve hypoxia tolerance in rainbow trout, potentially through its antioxidant properties and with possible sex-specific effects. These results have important implications for aquaculture, offering a nutritional strategy to enhance fish welfare and performance under climate-induced environmental stress.

**Acknowledgements**

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# HOW ACTIVATION SOLUTIONS AFFECT FERTILIZATION OF SIBERIAN STURGEON *Acipenser baerii* EGGS ?

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**Introduction**

The success of fertilization of sturgeon eggs depends on the condition and maturity of the spawners (females and males), the procedure of controlled spawning and incubation of the eggs. It is important to have ichthyological experience and the ability to apply modern methods of sturgeon reproduction biotechnology, considering the analysis of gamete quality and the use of optimal activation solutions. Sturgeon spawning can be carried out at optimum levels if all the above factors are considered.

**Material and Methods**

The present study was conducted with six male and two female Siberian sturgeon *Acipenser baerii* to test and optimise fertilisation using five activation solutions. Semen collected from 6 male Siberian sturgeon was used to fertilise Siberian sturgeon eggs using five experimental activation solutions: AS1, AS2, AS3, AS4, AS5 which differed in composition, pH and osmolality. At 4 hours after activation, the degree of fertilisation was analysed and the percentage of alive normal and abnormal embryos was determined.

The SCA semen analysis system (Microptic, Spain) was used for semen analysis. The following parameters were analysed in semen activated with the five activation fluids mentioned above: sperm motility (MOT; %), velocity of curvilinear movement (VCL; μms-1), ALH, PRG, LIN, BCF.

**Results**

The highest percentages of sperm motility and curvilinear velocity were observed in the group activated with the AS2 solution, while the lowest percentages were observed in the group activated with the AS3 solution. The highest percentage of fertilised eggs 4 hours after activation was observed in the group activated with AS1 and AS2 solutions, and the lowest in the AS5 activation solution. The presented research results showing the use of optimal fertilization solutions can provide valuable guidance for breeders involved in the reproduction and hatching of sturgeon

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# OVERVIEW OF PLASTIC AQUACULTURE EQUIPMENT USED IN NORWEGIAN SEA BASED FISH FARMS

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## Introduction

Most Norwegian fish farms consist of plastic (polymer) structures such as floating platforms made of polyethylene pipes, nets made of nylon yarn, and moorings of synthetic ropes. During recent years, discarded aquaculture structures are increasingly considered a resource for reuse or recirculation of materials. Estimates from 2017 indicated that Norwegian Aquaculture facilities consisted of up to 192 000 tons of plastic components (Hognes & Skar, 2017; Sundt et al., 2018).

To establish sustainable circular business models, it is essential to understand which plastic resources, i.e. used equipment, that will be available during the next years. Thus, an early goal in the SirkAQ project (https://sirkaq.com/) has been to establish an overview of types and amounts of polymer materials in Norwegian fish farming structures.

## Materials and methods

This work includes an analysis of the amount, geographic location, composition, quality, age and condition of plastic components used in sea-based farms during 2022. It is limited to plastic components of large volumes, i.e. floaters, nets and mooring lines, and feed pipes due to their frequent replacement.

Nine in-depth interviews of suppliers of plastic equipment were the main source of information. Semi-structured interviews were performed with the goal of answering the following research question: How much plastic can be found in Norwegian fish farms at sea, and what properties do they possess that are important to consider when deciding on their future use?

In the analysis, biomass reports (https://www.fiskeridir.no) and literature (McIntosh et al., 2022) were used to establish the number and geographic location of farms and floaters used in 2022.

## Results

We have estimated that a total of 5800 plastic fish pens could be found in Norwegian fish farms during 2022. About 91% of Norwegian fish pens were made of mainly plastic materials. There were 823 active fish farms along the Norwegian coastline, also including a minority of traditional steel cages and new types of fish farms (Føre et al., 2022).

Norwegian fish farms consisted of about in total 230,000 tonnes of plastic during 2022. The total amount of plastic in floating collars and bottom rings was about 175,000 tonnes. There were about 30,000 tonnes of nets in use, 21,000 tonnes of ropes in the mooring systems, and 3,300 tonnes of feed hoses. The analysis shows that over 80% of the mapped plastic was Polyethylene (PE), of which 66% was virgin HDPE 100. Furthermore, we found 10% Nylon, 6% polyolefins in the form of PE/PP, 3% PE/PET, and 1% UHMWPE. There are also smaller amounts of Polypropylene (PP).

The typical practical lifetime is around 15 years for cage collars, about eight years for nets (including storage between use), 10 years for mooring lines, and on average 1.5 years (one production cycle) for feed pipes. The plastic materials in discarded equipment are often in good condition.

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**Acknowledgement**
The work is done as part of the project "SIRKAQ" which is founded through the Norwegian Government "Green Platform Programme" (project no. 340887), with funding from the Norwegian Research Council, Innovation Norway and SIVA, in addition to funding from the Norwegian Retailers' Environment Fund.

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# MONITORING LOADS AND MOVEMENTS OF SUGAR KELP (*Saccharina latissima*) CULTIVATION LINES IN AN IMTA FARM

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## Introduction

Integrated multi-trophic aquaculture (IMTA), involves farming of multiple marine species at different trophic levels at the same aquaculture site. In the AURORA IMTA project, integrated aquaculture of Atlantic salmon (*Salmo salar*) and the brown macroalgae called sugar kelp (*Saccharina latissima*) is realized and developed at a full-scale sea-based facility in the North of Norway. Possible benefits of IMTA are currently investigated along with developing robust solutions for both biological production and structural design The main idea is that the kelp can benefit from absorbing dissolved inorganic nutrients from the salmon farming. In addition, their joint production may be an effective shared use of sea area.

A part of the current RnD-work includes developing knowledge to ensure a safe and effective design of such IMTA-farms. Currently there is little knowledge on expected loads from waves and currents (hydrodynamic loads) on seaweed cultivation lines (Endresen et al., 2019; Kool et al., 2022), and seaweed weight (hydrostatic loads). This results in application of unnecessarily high safety factors in design, which may result in sub-optimal and overly expensive farm designs. In addition, increased knowledge of possible displacements and movements of cultivation lines is of great interest, as this may affect growth (through e.g. light, temperature and water quality) and lead to line entanglement and seaweed abrasion.

## Materials and methods

Five cultivation lines with sugar kelp have been instrumented with two depth sensors and a load chackle each from April 25$^{th}$ to July 10$^{th}$ 2024. In addition, the mass density of the kelp was measured once or twice a month to facilitate weight calculation.

Initial numerical analysis indicated that cultivation lines were horizontal at high hydrodynamic loads, while they were sagging in calm water. Based on these findings, the experiment was designed to investigate the following hypotheses:

1. The kelp line position will vary both horizontally and vertically due to currents, waves and seaweed growth.
2. Hydrodynamic loads on cultivation lines can be found from load measurements during periods when depth sensors indicate horizontal line positions.
3. At maximum line sagging in calm water, the seaweed weight may be found from load measurements.
4. Mass density of seaweed can be found using Archimedes principle and measurements of weight and volume of seaweed and seawater

Since the kelp would easily decompose during unfavourable storing conditions, our tests were done in the field using newly harvested seaweed kept in fresh seawater.

## Results

All instruments, including five load chackles and ten depth sensors, worked well and produced continuous measurement data except during two periods of power failure. The findings supported all four hypotheses, also indicating that the developed methods for measuring of load, position and mass density worked well.

Vertical kelp line positions varied with several meters, and large sagging coincided with low measured loads. Peaks in load measurements coincided with horizontal lines (similar depth measurements over a line) positioned at relatively shallow waters.

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A method and procedure to measure mass density of seaweed (hypothesis 4) was developed and employed. The results showed that the kelp had a mass density of 920 ± 86 kg/m3, based on 14 separate measurements. This is lower than seawater, and it was observed that the kelp was floating (without visible air-bubbles). Previous literature has indicated a mass density of about 1100 kg/m3, i.e. slightly higher than water (Vettori & Nikora, 2017; Norvik, 2017). Possible reasons for this may be that the density may depend on site specific conditions like nutrients, kelp size and carbon content, morphology (Buck & Buchholz, 2004) or different treatment and storing of the kelp after harvesting.

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# A STEREO VISION FRAMEWORK FOR FISH MONITORING IN AQUACULTURE

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## Introduction

The aquaculture industry is shifting from manual operations and experience-based reasoning towards a more objective, data-driven approach to meet growing global seafood demand and support the expansion of salmon farming into larger and more exposed offshore sites. This shift is driven by the integration of intelligent sensors, mathematical models, and decision support or autonomous systems across production stages, aiming to increase productivity, enhance sustainability, and improve fish welfare. This study presents a stereo vision framework for automated fish monitoring in finfish aquaculture, enabling tasks such as fish detection, tracking, behaviour identification, and size measurement. The proposed frame reduces the need for intrusive or labour-intensive monitoring methods, providing a more precise and efficient foundation for fish stock management.

This work was financed by the Research Council of Norway through the project: CHANGE – An underwater robotics concept for dynamically changing environments [1].

## Materials and methods

Stereo videos of salmon fish were collected from two separate experiments at SINTEF's industrial-scale research fish farm site, Korsneset [2], in September 2022 (P1) and March 2023 (P2). P1 aimed to investigate fish behavioural responses to intrusive objects with varying appearance. A central structure, equipped with a stereo camera (comprising two synchronized Lucid TRI032S-CC GigE cameras) on top and two Ping360 sonars (one on top and the other on the bottom), was decorated in six different configurations varying in shape, size, and colour. The average fish weight during P1 was 1 kg. P2 was designed to study the impact of Remotely Operated Vehicle (ROV) motion on fish behaviour. The ROV was equipped with the same stereo camera setup as in P1, and the average fish weight during P2 was 3.5 kg.

The proposed framework employs two independent YOLOv8 models: one dedicated to fish detection using Oriented Bounding Boxes (OBBs), and the other focused on anatomical landmark identification through integrated keypoint detection. The approach began with stereo camera calibration to correct lens distortions and establish spatial relationships between the left and right views. Two custom training datasets were then created by annotating fish with OBBs and labelling specific body parts of the fish such as the mouth and fins to train the respective models. During deployment, each stereo video frame is first split into a calibrated and aligned left-right image pair. The OBB detection model is then applied to both images to detect fish, with detections in the left image tracked using the ByteTrack algorithm [3]. The keypoint detection model is subsequently used to localise desired anatomical landmarks on each detected fish body. Hungarian algorithm [4] thereafter associates the tracked fish and their keypoints in the left image with corresponding detections in the right. Disparities are computed from these associations and used to estimate depth and 3D coordinates through the pre-determined calibration parameters and triangulation geometry. This spatial reconstruction enables further estimation of fish 3D swimming trajectories and motion patterns by continuously tracking positions of body parts across frames, as well as measuring fish length by calculating the distance between the mouth and central caudal fin keypoints.

## Results

Figure 1a illustrates keypoint tracking, where the centre of the OBB, mouth, gill, and caudal fin of an individual fish were identified and tracked. These keypoints were used to generate detailed 3D trajectories and motion patterns, as visualised in Figures 1b and 1c. The results revealed distinct motion patterns across different body parts: the caudal fin displayed the most dramatic speed variations, followed by the mouth, while the gills and OBB centers showed comparatively lower variability across all metrics.

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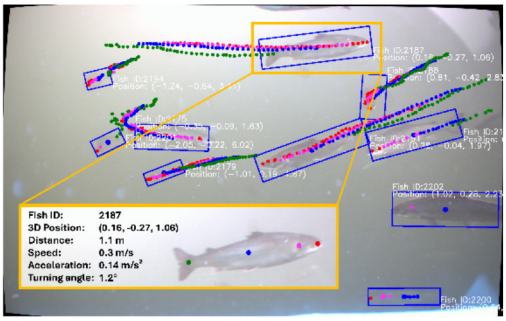


***Figure 1a:*** *Tracked fish illustrating detected keypoints and their 3D trajectories.*

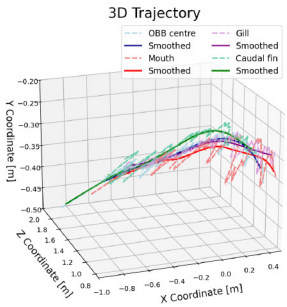


***Figure 1b:*** *3D trajectory of fish no. 2187.*

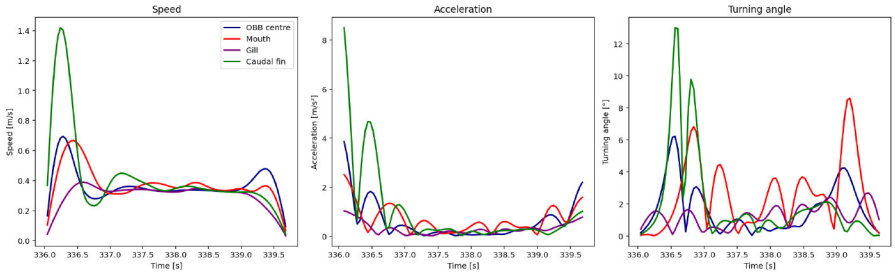


***Figure 1c:*** *Motion characteristics of fish no. 2187.*

Fish body lengths estimated from the stereo videos were 39 cm for P1 and 56 cm for P2. To validate these measurements, a weight-length relationship based on commercial farm data [5] was used to calculate reference body lengths of 41 cm and 63 cm for P1 and P2, respectively. The stereo video estimates closely match the reference lengths, demonstrating the reliability of our length estimation methodology and highlighting its potential for future applications.

**Conclusion and future work**

The proposed stereo vision framework, utilising OBB-based object detection and keypoint detection models, provides a robust, automated, and non-invasive solution for fish monitoring in aquaculture. Future developments will aim to integrate the two detection models into a unified end-to-end network to enhance processing speed. Additionally, extending the number of detectable keypoints will allow for capturing the contours and natural curvature of the fish body, providing a more accurate representation of its morphology for detailed sizing and behavioural analysis.

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# EVALUATION OF COMMERCIAL PHYTASE ENZYMES ON DIGESTIBILITY AND GROWTH PERFORMANCE IN FEED FOR *Sparus aurata*

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Phosphorus plays an important role in animal growth, especially for bone formation. In current feed, inorganic phosphorus (MCP, DCP) is commonly used to fulfill needs of fish. Phytic acid, is found in plant and constitutes an unused reservoir of phosphorus in feeds. Furthermore, phytic acid is an antinutritional factor affecting other nutrients such as amino acids, or minerals availability by binding them.

TECHNA tested 3 different phytases in feed deficient in phosphorus to evaluate their effects on digestibility and zootechnical parameters on Gilthead seabream (Sparus aurata), at the Hellenic Centre for Marine Research (HCMR). Phytase, named G, H and K for confidentiality comes from different phytase suppliers.

Six diets were designed for this experiment, three controls with varying level of phosphorus (0.3, 0.45 and 0.6% digestible phosphorus), designed to validate the phosphorus dose response. Three test diets with the same formula as the 0.3% digestible phosphorus value plus the addition of phytase. Phytase levels in feed were calculated based on supplier recommendation and therefore differ (1000 to 1500 FTU or u/g). All feeds were similar for 98% of their composition. Monocalcium phosphate was used for the 0,6% digestible phosphorus diet and replaced by calcium carbonate partially or fully to reach 0,45 and 0,3% feeds.

In HCMR facilities, 40 seabreams with an initial weight of 16.2 ± 0.04g were stocked in tanks filled with salt water at a temperature of 23-25°C. Each treatment was done in triplicate. The growth phase lasted 76 days and was followed by a two-weeks digestibility phase.

Phosphorus ADC was improved in diets containing phytase G and K compared to its respective control (Negative or 0.3%).

The results showed that the higher the phosphorus was, the better were the performances (FCR and final weight). Only phytase G had higher final weight but remain closer to negative control than intermediate control (expected growth).

| Treatment | Phosphorus ADC (%) | Protein ADC (%) | Fat ADC (%) |
|---|---|---|---|
| Negative control | $12.85 \pm 1.47^{c}$ | $92.15 \pm 0.95^{cd}$ | $74.86 \pm 0.73^{c}$ |
| Intermediate control | $49.34 \pm 1.90^{a}$ | $95.56 \pm 0.17^{a}$ | $80.48 \pm 1.07^{b}$ |
| Positive control | $52.80 \pm 2.14^{a}$ | $95.44 \pm 0.57^{ab}$ | $85.38 \pm 1.01^{a}$ |
| Phytase G | $24.94 \pm 1.82^{b}$ | $93.61 \pm 0.14^{bc}$ | $79.61 \pm 1.51^{b}$ |
| Phytase K | $26.74 \pm 0.54^{b}$ | $91.58 \pm 1.24^{d}$ | $82.27 \pm 1.83^{ab}$ |

Figure 2. Digestibility coefficient of feeds fed to Gilthead Seabream (*Sparus aurata*)

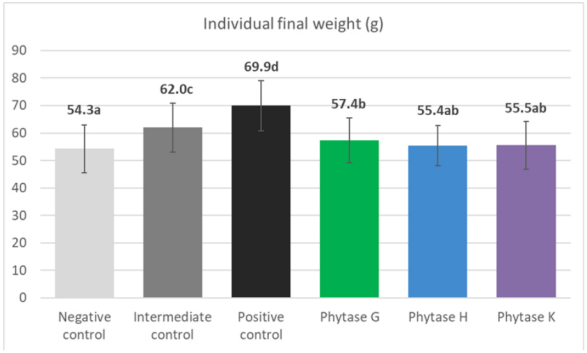


Figure 2. Final weight of Gilthead Seabream (*Sparus aurata*) fed the different diets

In conclusion, even if phytase were able to increase phosphorus digestibility, it was not sufficient to improve growth and FCR to the expected value. Digestible phosphorus valorization following this trial result was far from the supplier's recommendation (0.15% digestible phosphorus).

# BIODEGRADABLE CONTAINERS AS ALTERNATIVE FOR FISH SPERM CRYOPRESERVATION

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## Introduction

Nowadays, the most used fish sperm cryo-containers are straws made of polypropylene or polyvinyl chloride (PVC). They cannot be reused, making them waste with a high potential for polluting the environment. Thus, we aimed to assess the efficiency of hard-gelatin and hard-hydroxypropyl methylcellulose (HPMC) capsules as biodegradable alternative containers to plastic straws for fish sperm cryopreservation.

## Material and Methods

South American silver catfish *Rhamdia quelen* sperm samples (n = 12) were cryopreserved in 5% D-fructose + 5% powdered milk + 10% methanol diluted in a ratio 1:4 (sperm: cryo-solution). European eel *Anguilla anguilla* sperm samples (n = 12) were cryopreserved using P1 extender + 5% egg yolk + 10% methanol diluted in a ratio 1:9 (sperm: cryo-solution). Gilthead seabream *Sparus aurata* sperm samples (n = 12) were cryopreserved in NAM (non-activating medium) extender with 10% dimethyl sulfoxide (DMSO) after dilution in a ratio 1:6 (sperm: cryo-solution). European sea bass sperm samples (n = 10) were diluted in NAM at 1:5.7 (sperm: NAM), and 5 % DMSO was added. Sperm samples of all species, with motility over 60%, were individually cryopreserved in plastic straws, hard-gelatin, and hard-hydroxypropyl methylcellulose (HPMC) biodegradable capsules. All the containers were filled (silver catfish and seabream – 0.25 mL; eel and sea bass – 0.5 mL) and cryopreserved in liquid nitrogen vapor. The straws were thawed in a water bath (silver catfish 25 °C/10 s, eel 40 °C/13 s, seabream 60 °C/5 s, and sea bass 35 °C/ 15 s), and the capsules were thawed and dissolved into a plastic tube (15 mL) containing a previously warmed extender (5 mL- silver catfish 25 °C, eel and seabream 40 °C, and sea bass 35 °C) under shake in a vortex for 25 s.

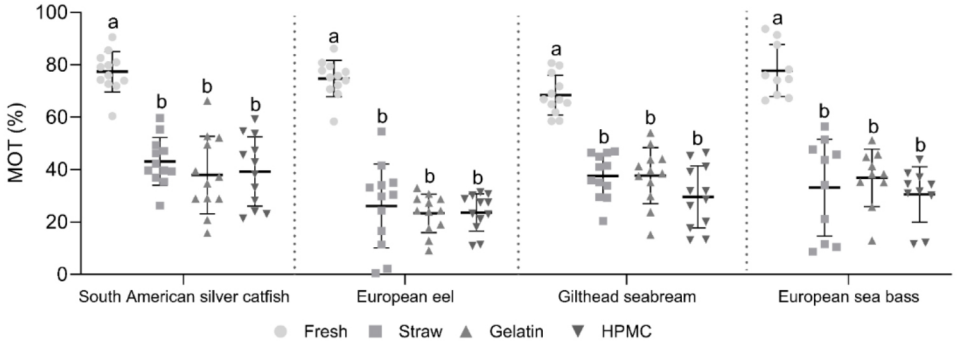


Figure 1. Fresh and post-thawed sperm motility (MOT) of South American silver catfish (n = 12), European eel (n = 12), gilthead seabream (n = 12), and European sea bass (n = 10) evaluated by CASA-Mot. The samples were cryopreserved in plastic straws, hard-gelatin, and hard-HPMC capsules. Values are shown individually, and horizontal lines indicate the means ± SD. Different letters indicate differences ($P < 0.05$; Tukey's test) between means.

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Total motility (MOT - %), curvilinear (VCL - μm/s), straight line (VSL - μm/s), and average path (VAP - μm/s) velocities were evaluated by CASA-Mot software. Rates of fertilization, hatching, and normal larvae morphology were determined using silver catfish post-thawed sperm. The alkaline comet assay was performed to quantify sperm DNA damage of eel, gilthead seabream, and sea bass. Tail-DNA (%) was evaluated using CaspLab software.

**Results**

In all four species, sperm cryopreservation led to a MOT decrease in all the storage containers (Fig. 1). Nonetheless, no significant differences in MOT were found between samples preserved in capsules and straws. A similar result was observed in sperm velocity parameters. Eel sperm cryopreserved in straws retained velocity values comparable to fresh samples, while those stored in gelatin capsules preserved their VSL and VAP post-cryopreservation. For sea bass, sperm stored in gelatin capsules exhibited higher velocities than those preserved in HPMC capsules.

There was no difference between straw and capsules in silver catfish fertilization, hatching, and normal larvae rates. Cryopreservation did not result in detectable DNA fragmentation in eel and sea bass sperm. Conversely, Tail DNA of gilthead seabream sperm cryopreserved in gelatin (10 ± 2%) and HPMC (11 ± 6%) capsules increased after thawing when compared to fresh (4 ± 3%) and straw-stored (4 ± 1%) samples.

**Discussion**

During cryopreservation, cells are exposed to high thermal and osmotic stress, mainly during freezing and thawing, causing damage to the cells (Mazur et al., 1972) and impairing sperm motility of all four species. However, the kinetic parameters of sperm samples cryopreserved in straws and capsules did not differ. It was noted that silver catfish samples cryopreserved using all three container types achieved fertilization rates of approximately 70%, a value considered high according to Beirão et al. (2019). We did not observe increased sperm DNA damage in European eel and sea bass after freezing and thawing. However, we observed a Tail DNA of 10 and 11% in sperm cryopreserved in gelatin and HPMC capsules, respectively. These results indicate low sperm DNA damage, and the oocyte could probably repair this level of damage during early embryogenesis.
Based on our results, hard-gelatin and HPMC biodegradable capsules can be an alternative to plastic straws for South American silver catfish, European eel, gilthead seabream, and European sea bass sperm cryopreservation.

**Acknowledgments**

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# DEVELOPMENT OF CHALLENGE TESTS TO SELECT SPERM SAMPLES FROM MEDITERRANEAN FISHES BEING RESILIENT TO CLIMATE CHANGE

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**Introduction**

Climate change impacts the marine environment, which faces acidification and rising temperatures. As key fishes in Mediterranean aquaculture rely on external fertilization, these environmental changes can impair sperm quality and reproduction. Therefore, we aimed to evaluate the effect of temperature and pH of artificial seawater (ASW) on the activation of sperm from different aquaculture Mediterranean species. Furthermore, sperm evaluation was used to select resilient males and to form a germplasm bank with climate change resistant samples.

**Material and Methods**

European sea bass, *Dicentrarchus labrax* (n = 30), gilthead seabream, *Sparus aurata* (n = 93), and Senegalese sole, *Solea senegalensis* (n = 116) sperm samples were collected at the Institute of Aquaculture Torre de la Sal (IATS - CSIC). In addition, samples from S. sole males (n = 16) were collected at the Institute of Agrifood Research and Technology (IRTA). Samples were obtained by abdominal pressure, diluted in species-specific extenders (NAM or Ringer solution) and transported at 4 °C to the Universitat Politècnica de València (UPV). At UPV, sperm motility was analyzed using a CASA-Mot system after activation with ASW. Only samples with more than 50% motility were used for further experiments.

Three challenge tests were tested trying to identify sperm samples that were resilient to environmental pH and temperature variations. The first one was the sperm activation using ASW at different pH (6.5, 7.2, 7.4, 7.6, 7.8, 8, 8.2, 9.5). The second challenge test was the sperm activation with ASW at different temperatures (seabream and S. sole: 4, 16, 22 °C; sea bass: 4, 12, 20 °C). The third one evaluated the combined effect of ASW pH and temperature: pH (7.8 and 8.2) x (seabream and S. sole: 4, 16, 22 °C; sea bass: 4, 12, 20 °C). Before activation, the samples were incubated (~1h) at the same temperature.

The challenge test combining seawater pH and temperature was the most effective for selecting resilient males. Therefore, sperm samples from sea bass (n = 10), seabream (n = 43), and S. sole (n = 10) were subjected to challenge test 3. Males were selected according to the variation in the MOT percentage obtained by applying variations caused by climate change (acidification and temperature increase) compared with the natural environment during their reproductive periods (pH 8.2; sea bass, 12 °C; seabream and S. sole, 16 °C). Males were selected considering the variations on sperm motility in comparison with values obtained under natural conditions. Different selection thresholds can be applied: up to 5% variation for a rigorous level, up to 10% for a less rigorous level, and up to 15% for S. sole samples.

**Results**

No practical differences were observed in the motility of the three species when challenge tests 1 and 2 were applied. However, in challenge test 3, it was clear that acidification and warming of the marine environment affected the sperm quality of the three Mediterranean species. The temperature increase impaired the sea bass (Fig. 1A) and S. sole sperm (Fig. 1B), and the decrease in ASW pH affected the MOT of sea bream sperm (Fig. 1C).

After applying the challenge test 3, 6 sea bass were selected at a 5% variation level and 7 at up to 10% variation concerning the MOT values obtained with the natural parameters of pH and temperature during the reproductive period. For seabream, 18 males at 5% variation level and 32 males at up to 10% level were selected. Only 1 S. sole male at the 5% and 2 at 10% variation levels were selected. At the 15% level, 6 breeders were selected.

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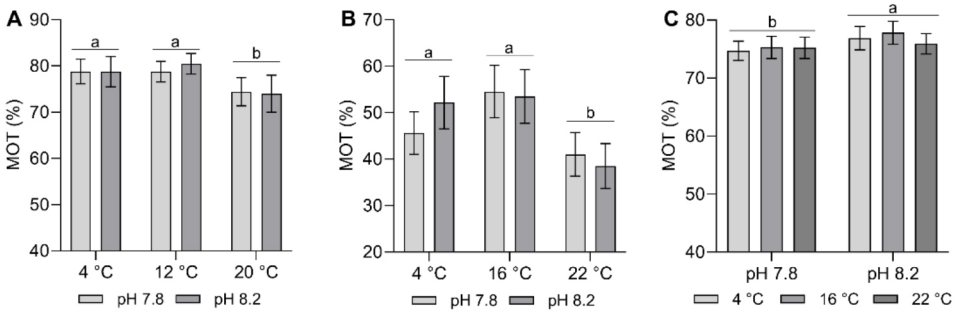


Figure 1. European sea bass (A – n = 10), Senegalese sole (B – n = 10), and gilthead seabream (C – n = 20) sperm motility (MOT - %) evaluated by CASA-Mot after activation using artificial seawater (ASW) at the same incubation temperature and pH adjusted to 7.8 and 8.2.

## Discussion

Environmental pH and temperature influence sperm motility (Alavi & Cosson, 2005). Sperm of some species can resist slight environmental acidification, as was observed in sea bass and S. sole in the present study. However, seabream sperm was impacted by acidification. Sperm activation in a more acidic environment may have caused a reduction in the membrane potential of sperm mitochondria since seminal plasma alkalization is necessary for sperm activation to occur (Alavi & Cosson, 2005). Increasing the incubation and activation temperature did not affect seabream sperm, while impaired sea bass and S. sole sperm motility. The sperm activation using above-normal temperatures leads to a decline in sperm motility, probably due to the limited antioxidant enzyme efficacy in mitigating oxidative stress (Dadras et al., 2017).

Alterations in pH and temperature driven by climate change impacted the sperm quality of three Mediterranean aquaculture species. The developed challenge tests showed to be a valuable technique for identifying males resilient to such environmental stressors, whose genetic material can be conserved in germplasm banks.

## Acknowledgements

This study forms part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana [GVA-THINKINAZUL/2021/012, -24, -42; Principal investigators: J.F. Asturiano (Universitat Politècnica de València), J. Pérez-Sánchez, and A. Gómez (CSIC), respectively].

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# MAPPING REGIONAL CHALLENGES IN ATLANTIC MARINE BIOTECHNOLOGY: A STAKEHOLDER-DRIVEN APPROACH TO INNOVATION AND SUSTAINABILITY

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**Introduction**

Marine biotechnology is increasingly recognised as a key approach to understanding and harnessing the potential of marine bioresources, while also supporting the development of tools and technologies to explore them. Emerging products, processes, and techniques have the potential to contribute significantly to the sustainable development of the blue bioeconomy. Ocean, marine biodiversity, and biomass hold vast untapped potential that marine biotechnology could help unlock, particularly in the context of global challenges such as food security, energy, and health.

The regions of the European Atlantic Area face both complex challenges and promising opportunities across a range of marine sectors. This underscores the need for a coherent, regionally harmonised strategy to ensure marine biotechnology advances equitably and efficiently across the Atlantic basin. Realising its full potential requires the alignment of knowledge gaps, improved technology transfer, and effective policy integration.

This study adopts a stakeholder-driven approach to explore marine biotechnology across the European Atlantic basin. The findings aim to inform initiatives that support innovation, competitiveness, and sustainable growth in the Atlantic marine biotechnology sector.

**Materials and methods**

Two rounds of participatory workshops were conducted in 2024 and 2025, totalling 12 workshops across eight Atlantic regions in Portugal, France, Ireland, and Spain. A diverse group of stakeholders representing the quadruple helix model (industry, academia, public sector, and civil society) ensured an inclusive, multi-stakeholder perspective.

Each workshop followed a structured approach, beginning with a pre-survey to identify key challenges and plan the organisation of working groups. During the workshop day, participants were introduced to three pre-defined Blue Biotechnology categories: (1) sustainable and resilient aquaculture, (2) omics and marine observation technologies, and (3) marine-derived products for industrial use. Depending on the number and expertise of attendees, they were divided into working groups or round tables. A collaborative affinity mapping approach was used. Discussions focused on identifying challenges and proposing potential solutions, using physical or digital post-its for input collection.

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The collected data was analysed into specific challenges and potential solutions. A total of 307 challenges were reported, all under the three thematic areas. These were further mapped using the PESTEL framework (Political, Economic, Social, Technological, Environmental, and Legal).

**Results and discussion**

Across the two years of stakeholder engagement, a total of 307 challenges were identified (153 challenges in 2024 and 154 in 2025), predominantly concentrated in the Technological (36%), Environmental (21%) and Economic (20%) domains of the PESTEL framework. The challenges reflect key technical barriers such as inefficiencies in extraction processes, scaling difficulties, limited knowledge transfer and weak industry-academia collaboration, and gaps in circular economy implementation. Political, Social, and Legal domains represented 23% of the total, highlighting licensing and regulatory complexities, funding constraints, stakeholder engagement gaps, intellectual property (IP) concerns and social acceptance issues.

The challenges identified were distributed across three priority areas (1) sustainable and resilient aquaculture (46%), with emphasis on climate-adaptive practices, circular approaches, sustainable feed alternatives, and diseases management. (2) Omics and marine observation technologies faced challenges (26%) around data integration, technological readiness, and ensuring interoperability of systems for comprehensive monitoring. (3) Industrial use of marine-derived resources (28%) identified needs related most with reproducibility, standardization, high production costs, regulatory approval, and market acceptance, underlining the critical gap between innovation and commercialization.

Stakeholders consistently identified two critical vectors across the three thematic areas: (a) the need for scientific and technological advancements, particularly in scaling-up processes, circular biomass valorisation, and technological readiness; and (b) enabling non-technological elements such as regulatory clarity, IP protection, streamlined approval pathways and market acceptance.

**Conclusions**

This study highlights the complex and region-specific challenges facing the development of Marine Biotechnology in the European Atlantic Area, emphasising the need for integrated policy and innovation frameworks. Stakeholder insights, when combined with strategic policy analysis, provide the foundation for cross-border actions to enhance competitiveness and foster sustainable innovation.

Future work will benchmark the identified needs and challenges against the EU Blue Bioeconomy Roadmap and the objectives of the European Green Deal to evaluate their alignment and strategic relevance. This will support the development of high-impact interventions that can accelerate transformation of marine biotechnology into a cornerstone of a sustainable and resilient Atlantic blue bioeconomy.

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**Acknowledgments**

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# THE EFFECTS OF TEMPERATURE IN EARLY DEVELOPMENT STAGES IN THE LIMPET *Patella ordinaria*

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## Introduction

Patellid limpets represent important fishery, gastronomic, cultural, and ecological resources in the Macaronesia region. Recently, the limpet *Patella ordinaria* has experienced a severe population as a consequence of intensive anthropogenic pressure. Understanding and optimizing larval production is essential for both the success of aquaculture and the conservation of *P. ordinaria.* The goal of this study was to evaluate the resistance and resilience of early life stages of *P. ordinaria* to thermal stress.

## Material and Methods

Adult limpets were collected from Ponta do Garajau (Madeira) in February 2025. Assays were conducted in glass vials (10 mL) and glass beakers (500 mL) kept in water baths. The water baths were supported by the MOSS (Marine Organisms Stress Simulator) mesocosm facility (MARE-Madeira), being applied seven temperature treatments (12, 15, 18, 20, 22, 24, and 26 °C). before to be used for the assays. The assays focused on the influence of the temperature over: 1) the embryological development (22h incubation), 2) the larval development (48h larval culture), and 3) accumulative embryological and larval development (72h).

## Results and Discussion

Results of this study suggests that optimal temperature for larval production occurs between 15 and 18 °C, with reduced production outside this range (Fig.1). This highlights a narrow thermal tolerance and the sensitivity of early life stages to temperature stress. This study provides new insights into the species' sensitivity, which will be crucial for future considerations in limpet aquaculture.

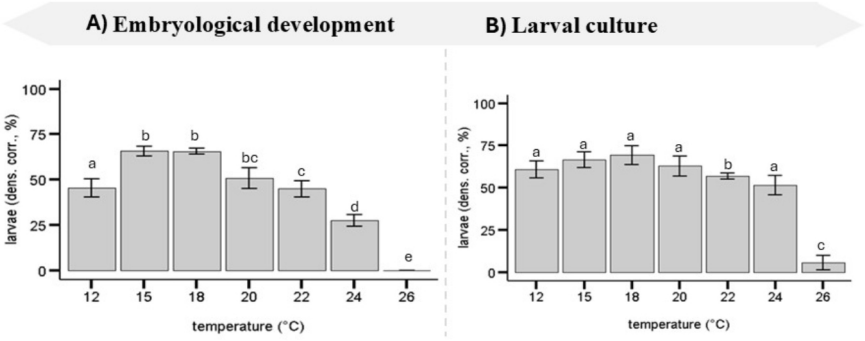


Fig. 1: The influence of temperature on larval production of *Patella ordinaria.* A) Ratio of viable larvae corrected by density during incubation. B) Ratio of viable larvae corrected by density during larval culture. Bars indicate average SD, and different letters indicate significant differences among the temperature ($p < 0.05$).

# DOSE-DEPENDENT EFFECT OF A BLEND OF NUCLEOTIDES ON ZEBRAFISH (*Danio rerio*) GROWTH PERFORMANCES

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## Introduction

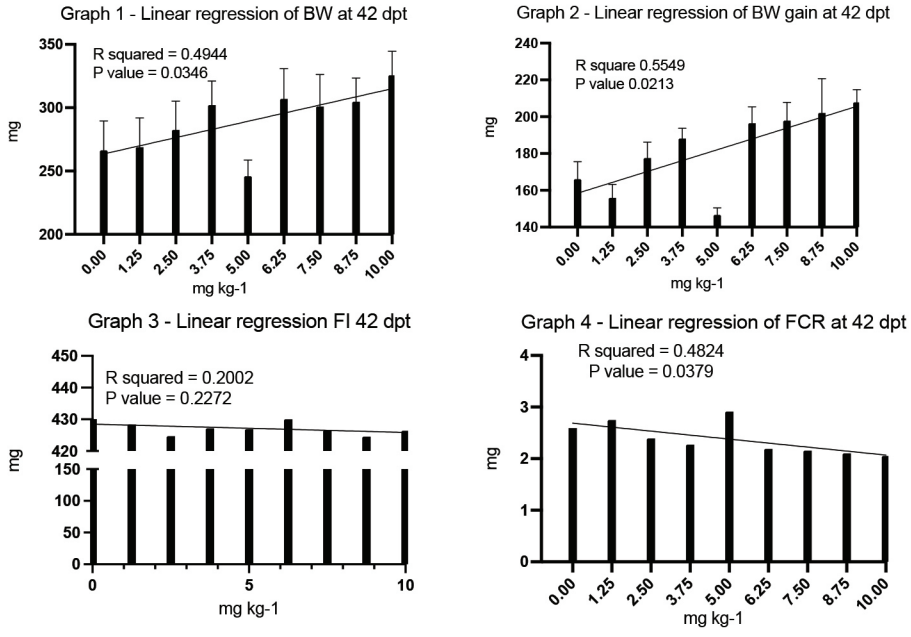
Challenges such as disease and environmental stressors represent a relevant threat to fish welfare and farm economic sustainability. Prophylaxis and good farming practices are important strategies to ensure fish welfare in farm conditions and reduce economic losses. Correct fish nutrition and feeding protocols are also key actions for a holistic approach to modern aquaculture production.

β-glucans, Mannano Oligo-Saccharides (MOS), antioxidants and other functional feed ingredients are more and more used in aquaculture. Unfortunately, their solely feed-inclusion is not sufficient to improve fish health and welfare and an appropriate combination of these ingredients is still necessary. This is also the case of nucleotides, a category of molecules characterized by different bioactivity and functions. Nucleotides are essential components of DNA and RNA and are involved in various physiological processes, including growth and immunity (Hossain et al., 2020).

The aim of this study was to investigate the dose-dependent effects of the feed inclusion of a specific blend of nucleotides, specifically formulated for aquaculture application, on fish growth performances. Zebrafish (*Danio rerio*) was used as animal model.

## Materials and methods

The study was carried out at the Zebrafish Facility of the Department of Veterinary Science, University of Pisa (Italy). Protocol and experimental design were approved by the Ethics Committee for Animal Welfare and Use of the University of Pisa and by the Italian Ministry of Health (code: B290E.N.LN2.).

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One hundred-eighty fish (102,3±26,43; m±ds) were uses to carry out the trial. The fish were divided into nine dietary treatments (20 fish per treatment), each one characterized by an increasing inclusion level of a specific bleand of nucleotide (Nucleo+ Marfeed, Miavit GmbH, Essen), as follow: 0, 1.25, 2.5, 3.75, 5, 6.75, 7.5, 8.75 and 10 g/kg. Water temperature was set at 28 °C, photoperiod was 12/12 h L/D and the feed was distributed *ad libitum,* 4 time per day (at 8, 11, 14 and 17), following the "five minutes rule" described by Lawrence (2007).

On days 0, 14, 28 and 42, each fish was slightly anesthetized (MS-222 Sigma, St. Louis, USA), photographed for further individual identification and weighted. Individual indentification was performed as already described by Fronte et al., (2021). Furthermore feed intake (FI) was measured, BW gain (BWg) calculated as difference between two following time points, and Feed Conversion Rate (FCR) calculated as FI/BWg.

The linear regression of final BW, BW gain, FI and FCR was performed to investigate the dose-reaponse effect of the considered treatments and parameters.

**Results and Discussion**

Excepted for FI, all the other considered parameters showed a significative dose-dependent effect. In detail, at the end of the experiment fish BW (Graph 1) was linearly enhanced (R squared 0.4922; P = 0.0346) by the dietary supplementation with the considered blend of nucleotides. Only exception was observed for the fish treated with 5 mg $kg^{-1}$ of nucleotides, which reached the lowest BW, with that not following the trend showed by the other groups. Similarly, also BWgain (Graph 2) linearly increased (R squared 0.5549; P = 0.0213). As already mentioned, FI (Graph 3) did not responded to the dietary supplementation (R2 0.2002; P = 0.2272), while FCR (Graph 4) linearly decrased according to the nucleotides supplementation (R2 0.4824; P = 0.0379).

In a previous study (Licitra et al., submitted for publication) carried out on a transgenic zebrafih model (*Tg(mpeg1:EGFP*)) an immunity enhancement effect was described (Licitra et al., submitted for publication). In present study, the same blend of nucleotides showed also a significant positive effects on fish growth. This effects seems to be linearly correlated to the feed inclusion rate. As described by Hossein et al. (2020), also other authors have already observed similar effects. However, the results of the present study suggest not toxic effect even at the higher dosage (10 mg $kg^{-1}$), but also that already 3.75 mg kg-1 may ehert significant effects on fish growth.

**Conclusion**

The aim of the present study was to define the possible efficacy of a blend of nucleotides, specifically formulated for aquaculture application, and the most appropriate dosage, on fish growth performances. The results showed a linear enhancement of the growth performances, starting from a dosage of 3.75 mg $kg^{-1}$ of feed. Also, no toxic effects were observed at higher dosages, the highest tested dosage (10 mg $kg^{-1}$) showing the best perfomances. Therefore, the results suggest a signicant efficacy of the tested blend of nucleotides, without evidences of possible toxicity effects.

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# GROWTH PERFORMANCE AND INTESTINAL HEALTH OF RAINBOW TROUT *Oncorhynchus mykiss* PREVIOUSLY SUBJECTED TO INFLAMMATION WITH DEXTRAN SODIUM SULPHATE (DSS)

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Aquaculture production has undergone remarkable growth during the past few decades, and it will continue to rise in the coming years to meet higher demand for safe, healthy and convenient seafood products. A lower usage of finite marine-harvested resources has been a major trend in the aquafeed industry. Grain and oilseed by-products are promising sources of protein and energy for aquaculture feeds. However, high dietary inclusion levels of plant proteins have often been associated to detrimental effects on growth, feed efficiency, intestinal dysbiosis and immune response, threatening the profitability of major aquaculture species.

Feed solutions that enhance digestive and metabolic processes have shown to be crucial to make these new feed formulation scenarios work, while reduce the environmental impact of aquaculture and promoting fish welfare. In the scope of this sustainable approach, Mixscience (France) developed a specific Phytogenics based solution that helps to reduce the negative impact of challenging feeds.

The aim of this study is to examine the effect of graded levels of feed additive (PHY) on the zootechnical performance, nutrient digestibility and intestinal mucosal health in rainbow trout (*Oncorhynchus mykiss*), previously subjected to a standardized inflammatory event with dextran sodium sulphate (DSS).

The Trout trial includes 5 treatments.
- A positive control **(PC)** = optimal diet for the targeted fish
- A negative control **(NC)** with lower levels of digestible protein and digestible energy due to fish meal replacement by challenging ingredients derived from defatted soybean meal and sunflower meal, which tend to reduce digestibility and negatively affect intestinal integrity.
- 3 additional diets, based on the NC formula, supplemented with the tested product **(PHY)** at a dose of 50 **(PHY50)**, 100 **(PHY100)** or 200 **(PHY200)** g/Tonne of feed.

The trial comprised 3 experimental phases:
- Phase I: A chemically induced intestinal inflammation using a diet with 1% dextran sodium sulphate (DSS) on the overall fish population for 10 days
- Phase II: An experimental feeding of the 6 experimental feeds over 86 days, to assess their effect on the growth performance and restoration of intestinal health criteria
- Phase III: a measurement of apparent digestibility of nutrients

The results indicate very clearly that in trout, compared to an optimal feed (PC), a challenge feed (NC) in which part of the fish meal has been replaced by plant ingredients and stress inducers, induces negative metabolic reactions: increase in systemic inflammatory status, expressed at blood and fecal level, a decrease in nutrient digestibility **(Figure1).** This outcomes in lower feed efficiency, and performance of fish **(Table 1).** The negative impact of a challenging feed can be compensated by specific phytogenics that activate key metabolic activities to save energy and maintain overall resistance that limits dysbiosis and intestinal disorders under stressful conditions and helps maintain a healthy optimal feeding efficiency and an overall fitness of the host. The results consolidate the results obtained in 2023 with Seabream. The optimal dose demonstrated for seabream of 200 g/T of feed is confirmed for trout. A positive effect is observed at a lower dose of 100 g/T of feed. A dose of 50 g/T is not sufficient to have a positive effect.

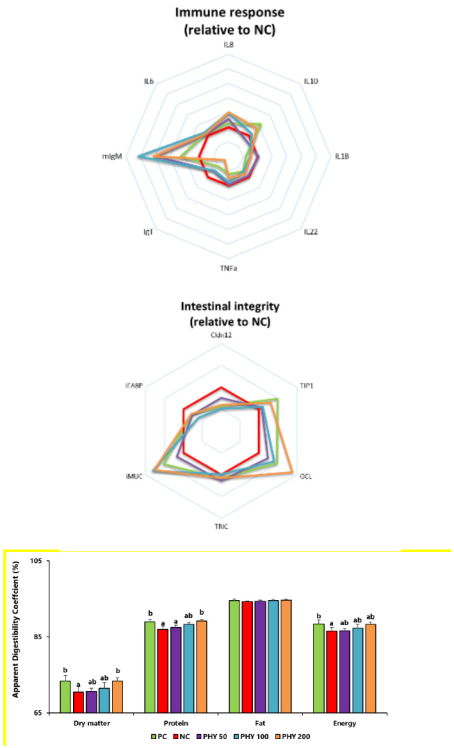
*(Continued on next page)*

**Table 1**. Growth performance after **86 days** of feeding (from day 10 to day 97)

| | PC | NC | PHY 50 | PHY 100 | PHY 200 |
|---|---|---|---|---|---|
| Survival, % | 99.4 | 99.4 | 100.0 | 100 | 100.0 |
| IBW, g | 33.6 | 33.6 | 33.6 | 33.7 | 33.6 |
| FBW, g | 160.0 $^{c}$ | 132.7 $^{a}$ | 135.5 $^{a}$ | 144.1 $^{b}$ | 152.5 $^{c}$ |
| SGR, %/d | 1.82 $^{c}$ | 1.60 $^{a}$ | 1.62 $^{a}$ | 1.69 $^{b}$ | 1.76 $^{c}$ |
| FCR | 0.89 $^{a}$ | 1.05 $^{c}$ | 1.03 $^{c}$ | 0.98 $^{b}$ | 0.93 $^{ab}$ |
| FI, %ABW/d | 1.35 $^{a}$ | 1.45 $^{c}$ | 1.44 $^{bc}$ | 1.41 $^{bc}$ | 1.39 $^{ab}$ |
| PER | 2.64 $^{c}$ | 2.21 $^{a}$ | 2.26 $^{a}$ | 2.38 $^{b}$ | 2.49 $^{b}$ |

Values are means ± standard deviation (n=4).
Values within a row with different superscripts, denote a statistical difference (P<0.05).

**Figure 1.** Results of the relative overall activity of the tested product at different doses on immune response, intestinal integrity and apparent nutrient digestibility compared to the negative control and the positive control



# INNOVATIVE PROTEIN CONCENTRATES FOR SUSTAINABLE AQUACULTURE: THE PROVEA RANGE

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The aquaculture industry is rapidly expanding and plays a crucial role in global food security. However, it faces significant challenges in terms of sustainability and nutrition due to a shortage of the marine protein sources.

To address these challenges, the Provea range focus on developing innovative protein concentrates from plant-based sources such as sunflower, faba bean, and pea. These concentrates, produced by Mixscience, a subsidiary of the Groupe Avril, aim to improve the nutritional composition and the digestibility of aquatic species, and to provide sustainable protein alternatives to formulate aquafeeds.

The Provea range offers a sustainable and nutritionally superior alternative for aquafeeds, contributing to the industry's growth and environmental goals. By leveraging locally sourced plant-based proteins, we can promote sustainable practices and support the development of a more resilient aquaculture sector.

This poster presents the results of our research on the nutritional composition, digestibility, and sustainability of the Provea range protein concentrates, as well as the benefits in fish farming.

**Nutritional Composition:** The protein concentrates have high protein content, ranging from 50-60%, which is essential for the growth and health of the fish. They also have a fat content of 3-6%, which helps in maintaining the overall health of the fish. The fiber content is moderate, less than 12%, ensuring good digestibility, and the moisture levels are around 8-10%, which helps in maintaining the stability and shelf life of the product.

**Digestibility:** The protein digestibility in trout is high, with values around 85-87%. This indicates that the protein concentrates are efficiently utilized by the fish, promoting growth and health. High digestibility is crucial for ensuring that the nutrients are absorbed effectively, leading to better feed conversion ratios and overall performance of the fish.

**Sustainability:** The project emphasizes reducing the carbon footprint by using locally sourced raw materials and adopting environmentally friendly processing methods. By supporting local agriculture and reducing dependence on imported fishmeal, the project contributes to the sustainability of the aquaculture industry. The use of plant-based proteins not only supports environmental goals but also promotes the development of a more resilient and sustainable aquaculture sector.

**Conclusion:** The use of these innovative protein concentrates in aquaculture feed offers several benefits, including improved optimization of formulation cost, animal nutrient digestibility, reduced environmental impact, and enhanced traceability of feed ingredients.

Continued research and development are essential to optimize the formulations and expand the applications of these protein concentrates in aquaculture. Future studies will focus on the impact on global fish performance and health

# OVERCOMING CHALLENGES IN IMPLEMENTING NOVEL FEED INGREDIENTS: INSIGHTS FROM CLOVER USAGE

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## Introduction

The pressing need to identify appropriate alternative resources to support aquafeed sustainability demands alternatives for resources that cannot directly be used for human food. Clover, a legume grown frequently as catch crop, was chosen as a novel non-food protein feed ingredient for the yellowtail amberjack (*Seriola lalandi*). The cultivation of clover grass can enhance soil quality, farm profits and the product is harvested in appropriate amounts to serve as a reliably available, sustainable, alternative protein ingredient for aquafeeds.

Its applicability in aquafeeds was tested

1. In a digestibility trial with the Yellowtail amberjack
2. In pellet production trials to test physical processing behavior.

## Material and Methods

Digestibility trials were conducted in a recirculating aquaculture system as described by Glencross et al. (2007). The reference diet was formulated to meet nutritional requirements of *S. lalandi* (Table 1) (IAFFD and pers. comm. Infinite Sea GmbH). The test diet was prepared by adding 3 parts clover gras protein concentrate to 7 parts of the reference diet mash on weight basis. Additionally, one test feed contained amylase to test its efficiency to support starch digestion in *S. lalandi* and one contained psyllium to test its effect on faeces properties. Each diet was fed to tank triplicates containing 20 fish.

Results presented will include digestibility of Clover grass, details of *S. lalandi* growth and health as well as rheological parameters of faeces.

## Pellet Production

A twin-screw extruder (ZK 20/40 P, Collin Lab & Pilot Solutions) was used to produce all experimental diets. The pellets were produced at a constant mass flow rate of 5 kg/h and with a 2 mm die. The screw speed (200, 400 rpm) and the temperature profile (Table 2) was varied during production.

The pellets were cut with a pelletizer (ELG 10, Econ GmbH) and dried continuously using an infra-red drum dryer (B.IRD Machinery Stricker GmbH). During measurement, the specific mechanical energy [SME] (Wh/kg), the pressure (bar) and the melt temperature (°C) were determined and documented using Fecon software.

## Pasting properties of pellets

The gelatinisation was determined using a Global Rheological Solution Viscosimeter (Australia) according to the ICC 169 method. The sample was mixed with water to form a 6 % starch suspension (dry substance) and placed in a sample beaker. Defined heating and cooling cycles were carried out for analysis. The measurement was carried out applying a heating cycle from 30 °C to 93 °C. A temperature of 90 °C was maintained for 20 minutes. The sample was then cooled to 50 °C and held again for 20 minutes. The stirrer speed was 75 rpm. The heating rate was 1.5 °C per minute. Global Rheological Solution software was used to determine gelatinisation temperature, highest observed viscosity, breakthrough viscosity, final viscosity and restoring viscosity.

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Table 1: Diet composition and corresponding calculated nutrient composition for test diets fed to *S. lalandi*. CGP = Clover grass protein.

| Ingredient (g/kg) | Control | Amylase | Psyllium | CGP 30/70 | CGP 30/70 + Amylase | CGP 30/70 + Psyllium |
|---|---|---|---|---|---|---|
| **Fish meal** | 500 | 500 | 500 | 350 | 350 | 350 |
| **Wheat gluten** | 150 | 150 | 150 | 105 | 105 | 105 |
| **Clover grass protein (CGP)** | | | | 300 | 300 | 300 |
| **Faba bean protein** | 80 | 80 | 80 | 56 | 56 | 56 |
| **Fish oil** | 80 | 80 | 80 | 56 | 56 | 56 |
| **Canola oil** | 30 | 30 | 30 | 21 | 21 | 21 |
| **Wheat** | 129,8 | 129,77 | 109,8 | 90,86 | 90,83 | 70,86 |
| **Taurine** | 10 | 10 | 10 | 7 | 7 | 7 |
| **Vitamin/Mineral Premix** | 20 | 20 | 20 | 14 | 14 | 14 |
| **Yttrium** | 0,2 | 0,2 | 0,2 | 0,14 | 0,14 | 0,14 |
| **Amylase** | | 0,03 | | | 0,03 | |
| **Psyllium** | | | 20 | | | 20 |
| **Nutrient composition** | | | | | | |
| **DM%** | **89,66** | **89,66** | **87,88** | **89,72** | **90,36** | **88,58** |
| **Ash%** | **8,04** | **8,04** | **8,02** | **6,88** | **6,80** | **6,78** |
| **GE MJ/kg** | **20,71** | **20,71** | **20,39** | **19,78** | **19,33** | **19,00** |
| **DE MJ/kg** | **18,36** | **18,36** | **18,13** | **16,66** | **17,35** | **17,12** |
| **CP%** | **53,80** | **53,80** | **53,60** | **55,70** | **55,06** | **54,86** |
| **Dig CP%** | **48,74** | **48,74** | **48,56** | **50,98** | **50,32** | **50,14** |
| **Lipid%** | **16,81** | **16,81** | **16,77** | **15,85** | **14,76** | **14,73** |

Table 2: Setting the temperature profile in the extruder (Z1, Z2, Z3, Z4 = Extruder Barrel °C, Z5= Temperature °C)

| | Zone 1 | Zone 2 | Zone 3 | Zone 4 | Zone 5 |
|---|---|---|---|---|---|
| Profile 1 | 40 | 50 | 90 | 110 | 130 |
| Profile 2 | 40 | 50 | 60 | 90 | 110 |

# CONTRASTING INTESTINAL IMMUNE AND EPITHELIAL RESPONSES TO WHEAT FLOUR AND WHEAT GLUTEN DIETS IN *Seriola dumerili*

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**Introduction**
The paradigm of feed formulation has shifted to address sustainability issues in aquaculture, primarily due to concerns about the environmental impact of traditional fishmeal-based diets (Tacon et al., 2022). However, this approach introduces unprecedented complexity in fish feeds, making it necessary to assess the cost-benefit of incorporating these new sustainable resources. As a result, plant-based proteins are increasingly utilised as replacements for fishmeal in aquaculture feeds, yet their impacts on intestinal immune, barrier, and functional properties remain unclear. We examined how replacing 50% of fishmeal with wheat flour (D1) or wheat gluten (D2) influences growth performance, as well as intestinal gene expression and physiology in juvenile *Seriola dumerili*.

**Materials and Methods**
Juvenile Seriola dumerili (~55 g initial mean body mass) were fed a premium control diet (CTRL) or experimental diets D1 or D2 for three months. Growth performance and feed efficiency were monitored. Anterior intestinal tissue was sampled for Ussing chamber measurements (Fuentes et al., 2025) of transepithelial resistance (Rt) and short-circuit current (Isc). Gene expression of immune-related (infg, il34), transport (slc15a1, slc15a2, slc15a4), and integrity-related (vil1, tjap1, tjp1a, tjp1b, cldn11, cldn12) markers was quantified by qPCR.

**Results**
Both D1 and D2 significantly reduced final mean weight, specific growth rate (SGR), and feed efficiency compared to CTRL ($p<0.001$), with no significant difference between D1 and D2 (Fig. 1). Ussing chamber assays showed that anterior intestinal Rt was significantly lower in D1 and D2 versus CTRL throughout the assay ($p<0.0001$), indicating impaired barrier integrity. Similarly, Isc was significantly different in D1 ($p = 0.0059$) and D2 ($p = 0.0145$) compared to CTRL, reflecting decreased active ion transport and the onset of a predominant secretory process (Fig. 1). At the molecular level, D1 significantly increased *infg* ($p = 0.0086$), while downregulating *vil1* ($p = 0.0002$) and *cldn11* ($p = 0.03$), indicating inflammation and epithelial remodelling. D2 also significantly decreased *vil1* ($p < 0.0001$) but increased *tjp1a* ($p = 0.0144$), while reducing *Slc15a2* ($p = 0.0333$) and *il34* ($p = 0.0245$), suggesting impaired immune homeostasis without overt inflammation. Notably, in both D1 and D2, *slc15a4* showed a non-significant tendency to increase, which may reflect a compensatory response to epithelial stress or altered immune signalling.

**Discussion**
These findings highlight distinct intestinal and physiological responses to diets containing wheat flour and wheat gluten. Both diets impair growth and feed efficiency, and reduce intestinal barrier function and active transport, indicating functional compromise of the mucosa. D1 triggers strong pro-inflammatory and epithelial remodelling responses, likely caused by fibre and antigenic components, which compromise barrier integrity. In contrast, D2 effects suggest a subtle decrease in IL34 and macrophage support, without causing overt inflammation. The contrasting regulation of SLC15 transporters and tight junction genes emphasises the complex interactions between diet composition, epithelial integrity, immune surveillance, and functional outcomes in the fish gut.

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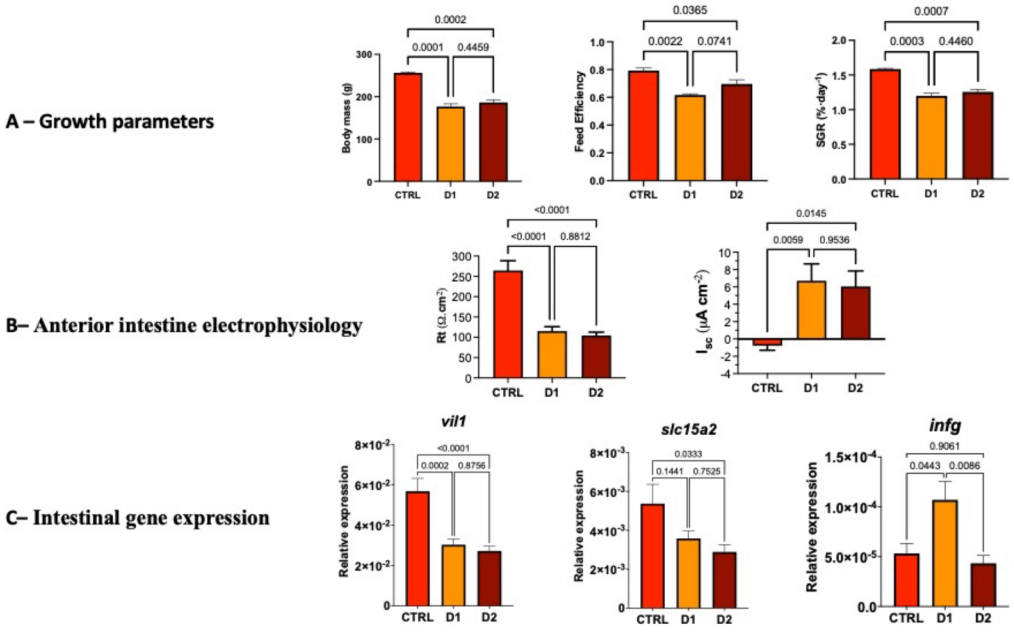


**Figure 1**. Effect of different diets on: A - growth parameters (final mean weight, feed efficiency (FE) and specific growth rate (SGR). B - anterior intestine electrophysiology (tissue resistance (Rt, Ω $cm^{-2}$), and short circuit current (Isc, μA $cm^{-2}$). C - intestinal expression of integrity- (*vil1*), transport- (*slc15a2*), and immune-related (*infg*) genes. Columns represent the average ±SEM (n=12); Diets: CTRL, D1, D2. Statistics were one-way ANOVA, complemented with a Tukey's post-hoc test.

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# EFFECT OF THE INCLUSION OF A BIOCHAR DERIVED FROM CHERRY TREEADDITIVE IN FEED FOR JUVENILE SOLE ON DIGESTIVE FUNCTIONALITY

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## Introduction

The intensification of aquaculture has driven the search for nutritional strategies that not only ensure the efficient growth of cultured species but also meet sustainability and functionality criteria (Quagliardi *et al*., 2024). In this context, the reformulation of feeds through the incorporation of alternative ingredients and additives with bioactive properties has taken a central role. One of the challenges associated with the partial or total replacement of fishmeal by plant-based sources lies in the adverse effects these ingredients can have on the digestive physiology of fish, due to the presence of antinutritional compounds that interfere with nutrient digestibility, alter intestinal morphology, and in some cases, affect feed efficiency and the quality of the final product (Seibel *et al*., 2022). In response to this issue, the use of functional additives capable of mitigating these limitations has garnered increasing interest. Among this group, biochars represent an emerging option with notable potential, derived from slow pyrolysis processes of lignocellulosic materials, such as agricultural residues, which give them a microporous structure and high adsorption capacity. These characteristics enable their application as modulators of the digestive environment by reducing undesirable metabolites and promoting more stable conditions in the gastrointestinal tract. Furthermore, it has been suggested that their inclusion in the diet may influence nutrient digestion and absorption, potentially generating indirect effects on protein and lipid deposition in muscle tissue, which is particularly relevant in commercially important species. In the case of sole (*Solea senegalensis*), a benthic species of high value in European aquaculture, knowledge about the physiological effects of biochar during early developmental stages is still limited, making it necessary to further explore its potential role as a functional additive in feeds formulated to improve digestive functionality and muscle quality during the juvenile phase.

In light of all these considerations, the main objective of this study was to evaluate the effect of the inclusion of cherry tree biochars in feeds with a high percentage of plantbased meals for juvenile sole on growth performance, muscle tissue proximate composition, and digestive functionality in juvenile sole.

## Material and Methods

To carry out the feeding trial, three experimental diets were formulated: a control diet (CT), based on plant meals; a B0.25 diet, supplemented with 0.25% biochar; and a B0.5 diet, supplemented with 0.5% of the functional additive. At the end of the feeding trial, muscle samples were collected to determine their proximate composition, and intestinal samples were taken to evaluate the activity of pancreatic secretion enzymes (trypsin, chymotrypsin, and total alkaline protease) and intestinal secretion enzymes (leucine aminopeptidase and alkaline phosphatase).

## Results and Discussion

The activity of digestive enzymes provides valuable information about the nutritional status of fish. Figure 1 shows the results obtained for digestive enzyme activity. In general, a decrease in the activity of pancreatic secretion enzymes was observed in B0.25 experimental group compared to the other groups. Meanwhile, fish fed B0.5 experimental diet showed the highest values of alkaline phosphatase activity, which could be associated with an increase in intestinal absorption capacity in these fish. However, although these results indicate a modification in the digestive functionality of the fish, further research is needed to draw more robust conclusions.

Likewise, the analysis of the proximate composition of the muscle in juvenile sole fed the different experimental diets showed a significant increase in lipid content in those fish that received the biochar-supplemented feeds, which could indicate a modification of lipid metabolism mediated by these functional additives.

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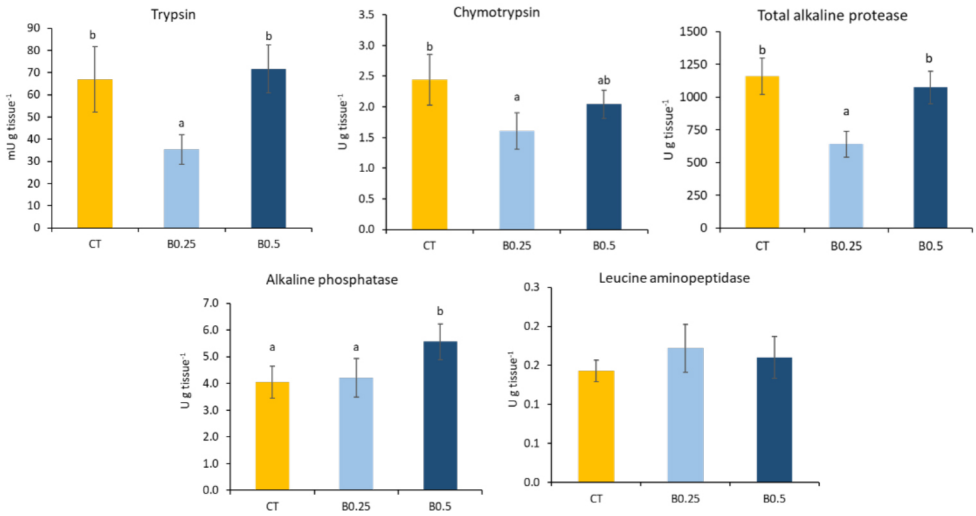


*Figure 1.* Digestive enzyme activities quantified in the intestinal extracts of *S. senegalensis*.

Acknowledge

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# EFFECTS OF DIETARY *Artemisia annua* SUPPLEMENTATION FOLLOWING AN IMMUNE CHALLENGE ON GROWTH, BLOOD PARAMETERS, AND MUSCLE LIPID COMPOSITION OF GILTHEAD SEABREAM JUVENILES (*Sparus aurata* L.)

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## Introduction

European aquaculture emphasizes new functional feeds as a strategy to maintain animal welfare and reduce reliance on antimicrobials. The use of medicinal plants holds strong potential as alternative to antibiotics and immunoprophylaxis in aquaculture. *Artemisia annua* is an annual herb species widely known for treating malaria (Soni et al., 2022), with reported benefits in fish including enhanced immune responses and reduced stress markers (Hoseini et al., 2022), although effects depend on dose and formulation. *A. annua* feed supplements are expected to improve fish health and growth, making them promising candidates for the development of functional and health-promoting feed additives in the aquaculture sector. Lipopolysaccharides (LPS) are commonly used to induce strong inflammatory responses in fish, making them useful for evaluating the efficacy of functional feeds in modulating immune and metabolic responses. The main objective of this study was to assess the effect of an *A. annua*-supplemented diet on *Sparus aurata* juveniles subjected to a LPS challenge focusing on growth performance, blood parameters, and muscle lipid composition.

## Material and methods

Juvenile gilthead seabream (n = 144) with an average weight of 47.18 ± 6.60 g were randomly distributed into 12 1-$m^3$ polyethylene tanks and assigned to either a commercial diet (C, control; 6 tanks), or the same diet supplemented with 30 g/kg of powdered dried *A. annua* leaves (A, experimental; 6 tanks). Six fish per tank were randomly tagged with microchips. Fish were fed 3% of their total biomass daily. After 37 days of feeding, three tagged fish per tank were intraperitoneally injected either with 5 mg/kg body weight of *Escherichia coli* LPS (3 tanks per diet), or an equivalent volume of phosphate-buffered saline (PBS; 3 tanks per diet), resulting in four experimental groups: C, A, CLPS and ALPS. Forty-eight hours post-injection, fish were sacrificed for intermediate sampling. Subsequently, the dose of *A. annua* in the A diet was reduced to 10 g/kg, and the same sampling procedure was repeated after 62 days (final sampling). At both samplings, blood was collected using heparinised syringes for parameter analysis, while muscle total lipid (TL) and fatty acid (FA) composition were determined. Survival and growth were monitored throughout the experiment.

Table I. Specific growth rate (SGR), blood parameters, and muscle total lipid (TL) content of *S. aurata* under the experimental treatments at both sampling points.

| | C | A | CLPS | ALPS | D | I | In |
|---|---|---|---|---|---|---|---|
| **Intermediate sampling** | | | | | | | |
| SGR (% $day^{-1}$) | 1.3 ± 0.1 | 0.6 ± 0.3 | 1.1 ± 0.1 | 1.0 ± 0.2 | * | | |
| Haematocrit (%) | 28.8 ± 9.2 | 18.1 ± 2.6 | 28.6 ± 5.9 | 15.8 ± 3.6 | * | | |
| TL (% dry weight) | 9.5 ± 3.5 | 14.7 ± 2.9 | 9.0 ± 1.3 | 12.7 ± 5.6 | | | |
| **Final sampling** | | | | | | | |
| SGR (% $day^{-1}$) | 1.3 ± 0.2 | 0.9 ± 1.3 | 1.3 ± 0.0 | 1.0 ± 0.2 | * | | * |
| Haematocrit (%) | 33.1 ± 5.5 | 18.4 ± 4.6 | 36.8 ± 5.0 | 26.5 ± 4.6 | * | * | * |
| Haemoglobin (mg/mL) | 68.6 ± 15.9 | 18.5 ± 6.5 | 80.0 ± 21.5 | 19.3 ± 6.1 | * | | |
| TL (% dry weight) | 15.8 ± 2.2 | 14.5 ± 3.0 | 15.8 ± 0.9 | 11.9 ± 4.4 | | | |

D, diet; I, injection; In, interaction. * Significant differences (Two-way ANOVA). Results are medians ± standard deviation (n= 9)

*(Continued on next page)*

**Results**

Fish survival (94-100%), TL, and FA composition of muscle was similar across all dietary treatments. However, specific growth rate (SGR) was primarily affected by the diet at both sampling points, while weight gain was affected by both diet and injection at the end of the experiment, with A diet and LPS injection showing the worst results ($p<0.05$). Additionally, viscerosomatic (VSI) and hepatosomatic indices (HSI) also decreased in fish fed the A diet ($p<0.05$). Regarding blood parameters, haematocrit and total plasma protein were markedly reduced in fish fed diet A at both samplings, while cholesterol decreased at the midpoint and haemaglobin at the end of the experiment (Table I). At the end of the experiment, haematocrit and triglycerides increased in response to LPS injection, while interaction between diet and injection had a significant effect on cholesterol, triglycerides, and haematocrit levels ($p<0.05$).

**Discussion and conclusions**

Although *A. annua* has been reported to improve growth in some species, it reduced growth and both VSI and HSI in our study. Similar results were reported for *S. aurata,* with *Artemia arborescens* supplementation, attributed to imbalances in nutrient digestion and absorption (Tzortzatos et al., 2024). Negative effects on growth and haematocrit were detected at the intermediate sampling, and although the dosage was readjusted, it was insufficient to mitigate them. The reduction in haematocrit and haemoglobin may indicate erythrocyte hemolysis and anemia, contrary to benefits reported in common carp (Hoseini et al., 2022). Medicinal plants like *A. annua* contain compounds that can induce both haemolytic and anti-haemolytic reactions in human erythrocytes (Sohou et al., 2024), though the role of artemisinin derivatives in fish remains unclear. At the final sampling, haematocrit increased in fish fed the A diet following LPS injection compared to PBS, possibly due to splenic contraction and the release of stored erythrocytes described during acute stress (Fazio et al., 2015). *A. annua* has been described to protect against lipid oxidation in common carp (Hoseini et al., 2022), but its direct effect on fish FA profiles is yet to be studied. In our work, TL and FA composition were similar across treatments. These findings suggest that *A. anua* supplementation may negatively affect growth and blood parameters in gilthead seabream. Further studies with varied doses under controlled conditions are needed to determine the optimal range for beneficial application, if any.

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# MONITORING THE EFFECT OF TANKS COLOUR ON THE DEVELOPMENT, COLOURING, AND BEHAVIOUR OF GILTHEAD SEABREAM (*Sparus aurata*)

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## Introduction

Improving the health of farmed species by defining optimal welfare conditions is a major current challenge in aquaculture. Many species form social hierarchies, and the dominant-subordinate relationship can cause stress, which may impact the animals' growth and well-being, directly affecting productivity. The gilthead seabream, *Sparus aurata*, can change its body colour in response to environmental changes and as a signal of behavioural strategies. This colour change may also influence aggressive behaviour (Batzina & Karakatsouli, 2014). In this study, the effect of tank colour on the growth, colouration and behavioural patterns of juvenile seabream was evaluated to better understand the species' comportment strategies.

## Material and methods

A total of 56 *Sparus aurata* juveniles (initial weight: 232.10 ± 50.90 g) were tagged with microchips and randomly distributed among eight 1 $m^3$-polyethylene tanks with two background colours: black (B) or white (W) (four tanks per colour). Fish were fed a commercial diet *ad libitum* five days per week. After 31 days, fish were subjected to colour sampling in 80 L filming tanks equipped with an underwater camera and a colour checker. Each fish was transferred either to a filming tank of the same colour (control) or of the opposite colour (experimental). Consequently, fish from black tanks were transferred to white tanks (B_W), whereas fish from white tanks were transferred to black tanks (W_B). Following a 5-minute video recording, fish were moved to a recovery tank and then returned either to their original (control) or experimental tanks, where they remained until the end of the trial (day 81). Still images were extracted from minutes 0-1 (T1) and 4-5 (T2). Colour data were standardized and RGB values (Red-Green-Blue model) were converted to HSV using the grDevices R base package (Rodriguez-Barreto *et al.*, 2019), allowing for the calculation of Hue (pure colour), Saturation (intensity) and Value (brightness).

Aggressive behaviour was recorded using underwater cameras during feeding sessions, both before and after colour sampling. The 2-min videos were analysed to quantify aggressive acts (charging and chasing, fighting, and circling). Fish survival and growth were monitored throughout the experiment.

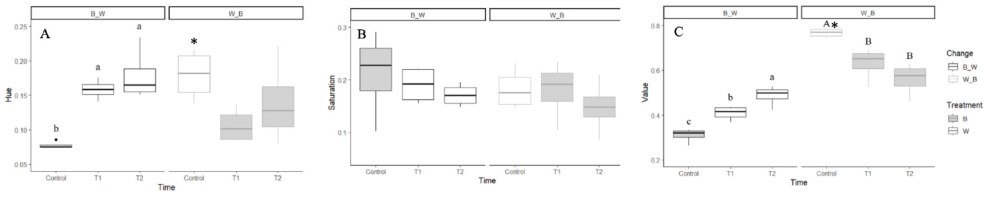


**Fig. 1.** Hue (A), saturation (B), and value (C) in control and two time periods (T1, 0-1 min; T2, 4-5 min). Results are medians ± SD (n= 4). B, black tanks; W, white tanks; B_W, fish transferred from black to white tanks; W_B, fish transferred from white to black tanks; ·, outliers; * indicates significant difference between B and W tanks within the control treatment ($p<0.05$, t-test); Different letters (a, b, c and A, B) indicate significant differences among control, T1, and T2 within the same colour-change group ($p<0.05$, one-way ANOVA).

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**Results**

Fish survival, growth, and colour intensity (saturation) were not affected by the tank bottom colour. However, in the control treatment, hue and value were lower in B tanks than in W tanks. After the tank colour change, fish from B_W showed higher hue values in both time periods (T1 and T2) compared to the control, whereas W_B fish showed no differences across treatments (Fig. 1). Value was lowest in the control and highest in T2 treatment for B_W. In W_B, the control showed the highest value. At T2, no significant colour differences were found between fish in B and W tanks. Lastly, the number of charges and chases showed no significant differences, and no fighting or circling behaviours were observed in any videos.

**Discussion and conclusions**

This study showed that juvenile *S. aurata* can be reared in either black or white tanks without negative effects on growth or survival. Fish colour intensity (saturation) was similar regardless of tank colour, although fish from B tanks were darker (lower hue) than those from W tanks, consistent with Wang et al. (2020) in red tilapia. Hue changed immediately after transfer from B to W tanks (B_W), reaching its final value within the first minute, whereas brightness (value) increased more slowly, stabilizing after 4-5 minutes. When fish were transferred from W to B tanks (W_B), brightness changed rapidly, whereas hue remained stable. Similar results were found by Wang et al. (2020) in red tilapia. No significant differences in hue or value were found between B and W tanks after five minutes (T2), suggesting that this period is insufficient for complete colour adaptation, although a trend toward adjustment was observed. The immediate response is likely mediated by the neuroendocrine system reacting to handling and environmental stress, whereas full colour adaptation may depend on slower, long-term physiological processes (Wang et al., 2020; MacLean, 2021).

Although background colour has been shown to affect aggressive behaviour in fish (MacLean, 2021), no differences in charging or chasing were found in this study, and neither fighting nor circling were observed—possibly due to the short recording time.

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**Acknowledgements**

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# A MACHINE LEARNING-DRIVEN EARLY WARNING SYSTEM FOR CRYPTOCARYONIASIS IN MARINE AQUACULTURE

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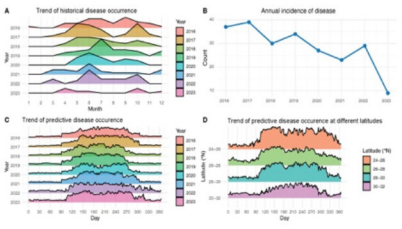
**Introduction**: Disease outbreaks, particularly cryptocaryoniasis caused by the ciliate *Cryptocaryon irritans*, pose significant barriers to sustainable marine fish aquaculture, undermining productivity, profitability, and biosecurity. Despite its impact, early warning tools for parasitic disease tools leveraging advanced technologies remain underdeveloped.

**Material and Methods**: We developed a machine learning (ML)-driven early warning system for cryptocaryoniasis, integrating seven years of outbreak surveillance data (n=429 events from 2016–2023) with 17 high-resolution oceanographic and environmental predictors selected for their influence on parasite life cycles across coastal China. Five supervised ML models: logistic regression (LR), support vector machine (SVM), random forest (RF), XGBoost (XGB), and artificial neural network (ANN), were trained using cross-validation and benchmarked in commercial open-sea cages and recirculating aquaculture systems (RAS).

**Results**: The RF model achieved the highest sensitivity (98.6%), with RF and XGB excelling over (F1 scores: 0.93 and 0.938, respectively), identifying stocking density, water temperature, salinity, pH, and novel predictors like silicate and nitrate as key risk factors. The resulting predictive engine was deployed as a web-based platform, freely accessible online via open-source environmental data, delivering weekly, spatially resolved outbreak forecasts. Field validation, across 12 open-sea cage events and weekly RAS monitoring, confirmed strong predictive accuracy (91.67% in sea cages; 87.5% in RAS), revealing seasonal and latitudinal trends in disease dynamics.

**Conclusion**: This study establishes a robust, scalable framework for real-time disease forecasting in marine aquaculture, adaptable to other aquatic pathogen-host species to support parasite surveillance and precision health management across diverse global aquaculture systems. While further validation with larger datasets and integration of pathogen and host data will enhance future models, this system provides a flexible foundation for advancing disease control in aquatic environments.



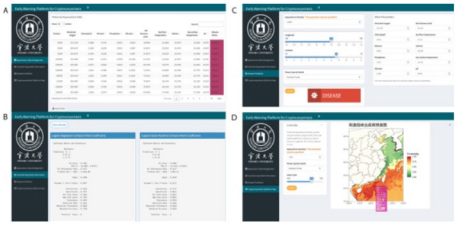


Fig. 1.- Temporal and spatial patterns in *C. irritans* occurrence.
Fig. 2.- Web-based cryptocaryoniasis prediction platform interphase
A) Historical trend, B) Annual incidence, C) Trend, D) Latitude.
A) Environmental data, B) Statistics, C) Disease prediction, D) Map

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# EVALUATION OF *Spirulina* AND *Azolla* AS PARTIAL REPLACEMENT FOR FISHMEAL IN FORMULATED DIET FOR KOI CARP (*Cyprinus carpio* var. *koi*) REARED IN AN AQUAPONIC SYSTEM

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Fishmeal is commonly used as a protein source in aquaculture feeds; however, there is a growing need to reduce its use due to sustainability concerns. This study evaluated the proximate composition (crude protein, lipid, fiber, ash, and nitrogen-free extract), palatability, attractability, and water stability of a formulated diet using *Spirulina* and *Azolla* as fishmeal replacements, as well as the growth performance of koi carp (*Cyprinus carpio var. koi*) cultured in an aquaponic system. Three different fishmeal replacement levels were tested: 30% and 50% replacement with *Spirulina* and *Azolla*. The parameters measured included weight gain rate (WGR%), specific growth rate (SGR%), survival rate (%), feed efficiency (FE), and feed conversion ratio (FCR). Water quality parameters—temperature, dissolved oxygen, pH, ammonia, nitrite, and nitrate—were monitored weekly. There were no significant differences ($P > 0.05$) in the proximate composition, attractability, palatability, water stability, or growth performance between the diets containing *Spirulina* and *Azolla* and the control diet. Similarly, there were no significant differences ($P > 0.05$) in the measured water quality parameters. The results indicate that *Spirulina* and *Azolla* can successfully replace up to 50% of fishmeal in the formulated diet for koi carp without negatively affecting growth performance or water quality in an aquaponic system.

# ONE STEP FURTHER TOWARDS A BETTER SOCIAL PERCEPTION OF SALMON FARMING IN CHILE PILOT OF A REGULATORY AND ENVIRONMENTAL COMPLIANCE PROGRAM

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Currently, public perception is critical for obtaining and maintaining a social license, as it directly influences societal support and acceptance. A positive public perception can strengthen community relations, while negative ones can generate conflict and resistance. Companies can develop and maintain a social license by demonstrating their commitment to transparency and communication, being open and honest with the community about their operations and impact. Likewise, they gain social legitimacy by respecting community norms and interacting with local stakeholders, providing reliable information and fulfilling commitments, thus building trust based on respect and mutual understanding. Specifically, this paper presents an experience with a regulatory and environmental compliance program, with a pilot program that included an academic third-party reviewer. The program aims to strengthen a commitment to sustainable practices and minimize the environmental impact of a salmon farming company's activities.

Traditionally, the Chilean salmon farming industry operated under a regulatory mechanism known as "command and control," where the environmental monitoring and regulatory support area, rather than a second line of defense, was an auditor of compliance with procedures or regulations. This auditing system is similar to that used by government authorities.

With the implementation of this pilot program, the goal is to identify and consolidate the different lines of defense in the company's environmental risk management and regulatory compliance. The above implies an original framework that emphasizes the transversality of environmental and regulatory risk management, directly including operational areas, as well as making it part of the company's strategic plan for sustainability. The challenge is to generate a true cultural change within the company. This means that all operational, administrative, and commercial teams, among others, are committed to this new system and understand their role as risk managers. They begin to understand themselves, measure themselves, and report on their risks, incorporating this new variable into their routine decision-making framework.

The progress made so far has included reviewing critical points of the company's compliance model that fall within Chilean regulatory and environmental aspects, as well as their relevance to the established risk needs and verifying the effective execution of the monitoring and improvement actions described in the compliance matrix.

The challenge for the future is to foster academic spaces that promote the training of new professionals and technicians, incorporating aspects of regulatory and environmental compliance, to generate competencies in both production and operational tasks, as well as support and governance, thus incorporating capabilities and value into companies in the face of the challenges and opportunities that arise, within a sustainable productive ecosystem connected to society.

# GENOMIC STRATEGIES FOR CLIMATE-RESILIENT MUSSEL FARMING: TOXIN ACCUMULATION IN *Mytilus galloprovincialis*

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## Introduction

The Mediterranean mussel (*Mytilus galloprovincialis*) is a cornerstone of European shellfish farming, particularly along the Atlantic coast, where Galicia (NW-Spain) alone produces approximately 220,000 tons annually. However, the increasing frequency of harmful microalgae blooms, caused by climate change, poses a major challenge to mussel aquaculture due to toxin accumulation and prolonged commercialization bans. In this study, we conducted genome-wide association studies (GWAS) on a large number of families, using a multispecies SNP Axiom Array (60K) to investigate the genetic basis of toxin accumulation following exposure to okadaic acid, the main diarrheic toxin. A chromosome-level genome assembly of *M. galloprovincialis* (1.8 Gb) was developed, and SNPs from the array were mapped to the annotated genome. Chromosomal regions showing significant association with toxin accumulation were analyzed for candidate genes, revealing a major quantitative trait locus (QTL) on chromosome 1. This locus appears to underlie the moderate heritability of the trait ($h^2 = 0.39$) and offers promising targets for selective breeding. These genomic tools represent a critical step toward more sustainable and climate-resilient mussel aquaculture.

## Materials and Methods

A population of 1138 mussel samples, belonging to the toxin accumulation group (APG group) described in Pino-Querido et al., 2015, was genotyped using a custom 60K multispecies SNP Axiom array, developed in collaboration with the University of Exeter. Genomic DNA was analyzed using standard GWAS pipelines, including quality filtering with PLINK 1.9 software (Chang et al., 2015). A chromosome-level genome assembly (1.8 Gb) was constructed using Oxford Nanopore (50x) and Illumina (120x) sequencing, resulting in 1,207 contigs (N50 = 2.4 Mb). The genome was scaffolded using a medium-density linkage map (11,540 loci in 14 LGs) and Omni-C data.

## Results

After quality control, a total of 22,499 high-confidence SNPs were retained, of which 8,891 were successfully aligned to the reference genome. Genome-wide association analysis revealed seven SNPs significantly associated with okadaic acid accumulation, supporting the presence of quantitative trait loci (QTL) with moderate heritability ($h^2 = 0.39$). The majority of these SNPs were localized on chromosome 1, delineating a key genomic region potentially involved in the trait; one SNP mapped to chromosome 4, while another did not align to the genome. A detailed annotation of the chromosome 1 QTL region showed that two of the significant SNPs are located within coding sequences of annotated genes. Further investigation of the intergenic region between these two SNPs revealed a tightly linked gene cluster, including candidate genes functionally associated with transport processes and immune response mechanisms.

## Discussion

This study highlights the utility of SNP genotyping arrays to identify genetic regions involved in toxin accumulation. The identification of QTLs offers valuable targets for selective breeding, aiming to develop toxin-resilient mussel lines and mitigate the socio-economic impact of harmful algal blooms on aquaculture.

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# NUTRIENT RECOVERY FROM SYNTHETIC AQUACULTURE EFFLUENTS THROUGH *Desmodesmus armatus* CULTIVATION

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## Introduction

The intensification of aquaculture production has raised increasing concern regarding the environmental impacts of nutrient-rich effluents, particularly those containing high concentrations of nitrogen and phosphorus compounds. A sustainable alternative for mitigating these impacts involves the use of microalgae, such as *Desmodesmus armatus*, which are capable of growing in wastewater while simultaneously assimilating excess nutrients. This study evaluated the growth performance of *D. armatus* in synthetic aquaculture effluents formulated with varying concentrations of carbon (C), nitrogen (N), and phosphorus (P), aiming to assess its efficacy as a bioremediation agent. Additionally, the approach aligns with circular economy principles by integrating nutrient recovery with the generation of high-value algal biomass, with potential application as aquafeed.

## Material and methods

The experimental design followed a two-factor factorial arrangement, investigating the effects of carbon (C) and macronutrient (nitrogen and phosphorus) concentrations on the growth of *Desmodesmus armatus* and its nutrient bioremediation performance. Four levels of C-glucose (4.2, 2.8, 1.4, and 0 mM) were combined with four dilution levels of nitrogen (N) and phosphorus (P) (100%, 67%, 33%, and 10%), with the highest concentrations corresponding to 8.6 mM N and 0.64 mM P. The synthetic effluent medium was based on the formulation proposed by Visvanathan et al. (2008), with modifications to C, N, and P concentrations and the inclusion of ammonia and nitrite as nitrogen sources. Bold's Basal Medium (BBM) was used as the control, resulting in a total of 17 treatments, each with two replicates. Cultures were conducted in 250 mL Erlenmeyer flasks containing 100 mL of working volume, maintained under orbital shaking (100 rpm), at 24 °C, with a 12:12 h light: dark photoperiod and an irradiance of 300 $\mu E\ m^{-2}\ s^{-1}$. The cultivation protocol consisted of an initial inoculation phase followed by three consecutive subcultures, each lasting 12 days. Daily monitoring included measurements of optical density at 720 nm and cell concentration determined by flow cytometry. On days 0, 6, and 12, additional analyses were performed to assess photosynthetic efficiency (Fv/Fm), pH, total ammoniacal nitrogen, nitrite-nitrogen ($NO^{2-}$), nitrate-nitrogen ($NO^{3-}$), phosphate-phosphorus ($PO_4^{3-}$), and total organic carbon (TOC) (2017). Statistical analysis included two-way ANOVA followed by Tukey's post hoc test. Principal Component Analysis (PCA) was employed to identify treatment effects and multivariate trends.

## Results and Discussion

Desmodesmus armatus exhibited growth under all tested conditions, with significant variation in response to the availability of organic carbon. Higher C-glucose concentrations (4.2 and 2.8 mM) consistently promoted algal growth that was greater than the control, resulting in increased biomass productivity regardless of nitrogen and phosphorus dilution levels. The principal component analysis (fig. 1) revealed a clear separation among treatments with different C-glucose concentrations (0, 1.4, 2.8 and 4.2 mM), indicating a strong influence of carbon availability on system performance. Dimension 1 (Dim1), which accounts for 44.7% of the total variance, distinctly separates the carbon-free treatments from those with higher concentrations (particularly 4.2 mM), while Dimension 2 (Dim2), explaining 36.5%, further discriminates intermediate groups. Variables associated with bioremediation, such as the consumption of ammonium (ConsNH4), nitrite (ConsNO2), and nitrate (ConsNO3), along with the initial nitrogen concentration (N0), were more closely related to treatments with low or no carbon supplementation. In contrast, variables linked to cultivation performance, including final productivity (Pr_final), final relative productivity (Pr_rel_final), and carbon consumption (ConsC), showed a positive association with treatments receiving higher glucose concentrations. These findings suggest that increased carbon availability enhances the growth of Desmodesmus armatus and is associated with changes in the consumption patterns of nutrients derived from aquaculture effluents.

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**Conclusion**

The results demonstrate that higher C-glucose concentrations significantly enhance the growth and biomass productivity of *Desmodesmus armatus*. Carbon supplementation also influenced nutrient consumption patterns, highlighting the key role of this microalga not only in optimizing high-value biomass production for potential use in aquafeed, but also in bioremediation through the efficient removal of nutrients from aquaculture effluents.

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# CHARACTERIZATION OF IMMUNE PARAMETERS IN ACELLULAR AND CELLULAR FRACTION IN MALE AND FEMALE OF ATLANTIC BLUE CRAB (*Callinectes sapidus*)

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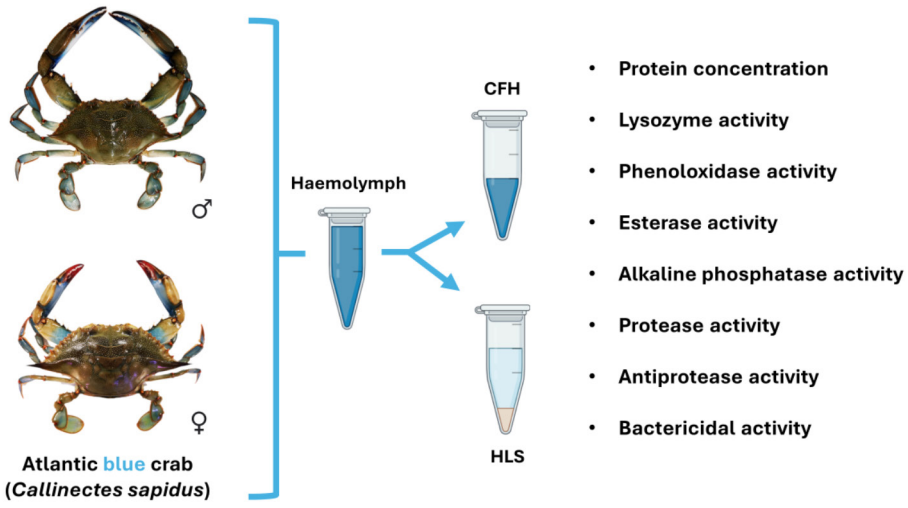
Despite their economic significance, brachyurans are relatively understudied among invertebrate groups compared to other decapods, with numerous fields remaining to be explored, including the immune response. Therefore, the aim of this study was to characterize several immune-related enzymes and activities of the Atlantic blue crab (*Callinectes sapidus*) in cell-free haemolymph (CFH) and in the haemocyte lysate supernatant (HLS), considering the gender factor and the suitability of measuring each activity in CFH or HLS. Data were expressed by millilitres of haemolymph or milligrams of protein to identify which of these units would be more robust and informative. For this, haemolymph from crabs of each gender was collected, and centrifuged to obtain CFH samples, whilst the cells were sonicated to get the HLS. Our results revealed that the protein concentration was higher in CFH compared to HLS. Considering milligrams of protein as units, phenoloxidase and esterase activities were lower in CFH regarding to HLS in both genders. Similarly, the lysozyme and phosphatase activities were lower in CFH compared to HLS but only in females. The values of lysozyme activity were higher in HLS of females compared to males. Studying millilitre of haemolymph as units, lysozyme activity exhibited higher activity in CFH compared to HLS in both genders, whilst phenoloxidase and esterase activities were higher in HLS, showing variations only in males. Interestingly, alkaline phosphatase activity was undetected in CFH male. Any variation was observed between the activities measured in both genders. Protease and antiprotease activities were higher and lower in CFH compared to HLS values, respectively, whilst any variation was observed between genders. Bactericidal activity against *V. harveyi*, *V. alginolyticus* and *V. anguillarum,* exhibited stronger values in HLS than in CFH, independently of gender, as well as against *P. damselae* in females. Considering gender factor, the bactericidal activity against *V. alginolyticus*, *V. anguillarum* and *P. damselae* was higher in HLS of females than in males. However, the bactericidal activity against *V. alginolyticus* and *P. damselae* was higher in CFH of males compared to females. Our results could contribute to gender selection in future brachyurans studies considering the variability between males and females due to their distinct biology. Additionally, we provide data regarding the baseline values of several immune-related activities, establishing the most appropriate units from the biological point of view.



# MEASURE OF PHAGOCYTIC ACTIVITY IN THE HAEMOCYTES OF THE ATLANTIC BLUE CRAB *(Callinectes sapidus)* USING LABELLED *Vibrio alginolyticus*

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## Introduction

Phagocytosis represents one of the most important cellular defence mechanisms in crustaceans, being essential for the elimination of pathogens and the activation of other immune processes (Sunjial et al., 2014). Despite its biological significance, the study of phagocytic activity in brachyuran crabs is still limited, with scarce information available for the Atlantic blue crab (*Callinectes sapidus*), a species of growing ecological and economic importance. Previous studies in crustaceans have employed several approaches to assess phagocytosis, often relying on microscopy or the use of yeast particles as model organisms. However, these techniques generally lack sensitivity and quantification accuracy. Flow cytometry offers a powerful tool to overcome these limitations, enabling a rapid and reproducible analysis of large numbers of cells, with the possibility to discriminate between cellular subpopulations. In this work, we aimed to develop and standardise a reliable method for the evaluation of phagocytic activity in Atlantic blue crab haemocytes by using fluorescein isothiocyanate (FITC)-labelled *Vibrio alginolyticus* as target particles and flow cytometry as the analytical platform.

## Materials and Methods

For bacterial preparation, *V. alginolyticus* was cultured in Tryptic Soy Broth supplemented with 1.5% NaCl at 25 °C until the exponential growth phase was reached. Bacteria were adjusted to $1 \times 10^9$ CFU $mL^{-1}$, inactivated with 3% formalin, and fluorescently labelled with FITC following a modified version of the method described by Yang et al (2020). Labelled bacteria were washed thoroughly and stored at –80 °C until use. For measuring the phagocytic activity, the method described by Esteban et al. (1998) was employed with some modifications. Haemolymph was extracted from six crabs using Alsever's solution with ethylenediamine tetraacetic acid (EDTA) as anticoagulant, and haemocytes were adjusted to $5 \times 10^6$ cells $mL^{-1}$. Each individual and time point, aliquots were prepared as positive (25 °C) and negative (4 °C) controls and incubated with FITC-labelled *V. alginolyticus* at a ratio of 50 bacteria per haemocyte. An additional aliquot from each crab was reserved for electronic transmission microscopic analysis. After centrifugation and incubation in the dark, extracellular fluorescence was quenched with trypan blue before analysis by flow cytometry (FACSCalibur, Becton Dickinson). A total of 10,000 events were recorded per sample. Forward scatter (FSC), side scatter (SSC) and green fluorescence (FL1) were collected. Phagocytic ability was defined as the percentage of haemocytes displaying green fluorescence, while phagocytic capacity was expressed as the mean fluorescence intensity (MFI) of positive cells. All experiments were carried out in triplicate and measurements were taken every 30 minutes from 0 to 180 minutes.

## Results

The method developed allowed a clear and reproducible quantification of phagocytic activity in Atlantic blue crab haemocytes. Haemocytes incubated at 25 °C (positive control) exhibited a progressive and significant increase in phagocytic ability over time, with the highest values observed between 120 and 180 minutes compared with earlier time points (0, 30, 60 and 90 minutes). In contrast, haemocytes incubated at 4 °C (negative control), where metabolic activity is reduced, did not show variations in phagocytic ability throughout the experimental period. Regarding phagocytic capacity, both positive and negative controls showed an increase in MFI over time compared with baseline values, but no variations were detected between the two conditions. These results suggest that the optimal time point for measuring this activity is between 120 and 180 minutes and also that cold reduces the metabolism of the cells and reduces drastically the phagocytic ability. Although, phagocytic

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capacity was not affected by cold treatment. The data obtained by flow cytometry were corroborated by transmission electron microscopy, which confirmed the internalisation of bacterial particles by haemocytes, providing complementary evidence to support the reliability of the method.

**Conclusion**

The present study establishes a reproducible and sensitive method for assessing haemocyte phagocytic activity in the Atlantic blue crab by combining FITC-labelled *V. alginolyticus* and flow cytometry. This protocol allows for the discrimination between phagocytic ability and phagocytic capacity, two complementary aspects of immune function, and contributes to the standardisation of methodologies for studying crustacean cellular immunity. The validation of results by electron microscopy further strengthens the robustness of the approach. Overall, this technique represents a valuable tool for the investigation of crustacean immune responses and could be adapted to other brachyuran and decapod species.

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# SEARCHING WELFARE BIOINDICATORS IN THE ATLANTIC BLUE CRAB *(Callinectes sapidus)* AFTER FISHING-STRESS FACTOR

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**Introduction**

The concept of animal welfare has traditionally been associated with vertebrate species, leaving aquatic invertebrates relatively understudied despite their ecological relevance and considerable economic value in aquaculture and fisheries. The lack of standardised methodologies for assessing welfare in these animals has hindered both the implementation of best practices and the establishment of regulatory frameworks. In recent years, there has been a growing recognition of the need to identify and validate species-specific biomarkers that may serve as reliable indicators of welfare status in aquatic invertebrates. Brachyuran crabs, due to their physiological and immunological characteristics, represent promising models in this regard. The Atlantic blue crab (*Callinectes sapidus*), an invasive species in the Mediterranean but also a species of commercial interest, provides an ideal experimental model to explore potential biomarkers of welfare. Parameters such as lactate and glucose have been commonly used as metabolic indicators in crustaceans (Albalat et al., 2009), while haemocyte-based assays, including viability, phagocytosis, and phenoloxidase activity, are frequently employed as markers of immune competence (Lightner et al., 2021). However, comprehensive studies combining these markers under controlled experimental conditions are scarce. This study aimed to assess a range of physiological, cellular, and humoral parameters in Atlantic blue crab over different time intervals following capture, in order to evaluate their potential as welfare biomarkers.

**Materials and Methods**

A total of 42 male of Atlantic blue crab (315 ± 62 g) were obtained from the fish market in Lo Pagán (Murcia, Spain) captured by traditional fishing methods. Crabs were transported in sealed dry containers, mimicking commercial conditions, and transferred to the marine facilities of the University of Murcia. Animals were placed separately in rack system tanks (35 L each compartment), each with a 10 cm sand substrate to allow burying behaviour. Animals were not fed during the experimental period. Haemolymph was collected from six crabs per time point (0, 6, 24, 48, 72 h, 7 and 14 days post-capture). From each 5 mL sample, 1 mL was centrifuged to obtain plasma for glucose and protein analysis. Lactate was measured *in situ* using a portable analyser (MARCA) with specific strips. The remaining haemolymph was diluted 1:3 in modified Alsever's solution, adjusted to 1,200 mOsm $Kg^{-1}$, and kept on ice. Haemocytes were washed and resuspended in Alsever's buffer for subsequent assays. Plasma glucose was quantified using a commercial hexokinase kit, and protein concentration was measured following the method described by Bradford (1976). Haemocyte counts were performed using an automated cell counter, and samples were adjusted to 5 × 106 cells $mL^{-1}$. Viability was assessed by flow cytometry using propidium iodide. Phagocytic activity (ability and capacity) was evaluated using FITC-labelled *Vibrio alginolyticus*. For humoral parameters, haemocyte suspensions were sonicated to obtain lysates, which were used to determine total intracellular protein concentration and phenoloxidase activity employing the method described by Gálvez et al. (2025).

**Results**

Metabolic indicators showed clear temporal variation. Lactate and glucose levels in haemolymph decreased significantly at all experimental times compared with baseline (0 h), indicating a recovery from fishing-related stress. Cellular markers revealed more complex dynamics. Haemocyte counts decreased only at 24 h, with the values stabilising thereafter. Haemocyte viability remained constant across all time points, suggesting that handling and transport did not compromise membrane integrity. However, phagocytic activity displayed marked fluctuations. In the case of phagocytic ability, the values decreased significantly

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# EFFECTS OF CHRONIC NITRATE STRESS ON *Litopenaeus vannamei* REARED IN INDOOR AQUACULTURE SYSTEMS

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## Introduction

According to FAO [1], *Litopenaeus vannamei* production is rapidly expanding (6.8 million tons) due to its palatability, high nutritional value, disease resistance, and rapid growth. To enhance environmental sustainability and biosecurity, indoor aquaculture systems are emerging, minimizing water discharge, but increasing total suspended solids, ammonia, nitrite, and nitrate. Although nitrate originating from the oxidation of ammonia and nitrite is less harmful, it is often disregarded in farm management. In chronic stress conditions, the safe level threshold for Whiteleg shrimp is set to 177 mg/L and [2], with its toxicity depending on factors such as concentration, salinity and duration of exposure [3]. With increasing aquaculture production, consumer concerns about animal welfare have grown. Physiological and morphological changes provide valuable insights into the effects of stress on shrimp welfare, as markers of compromised health [4]. Currently, limited information is available regarding *L. vannamei* chronic nitrate stress, with a lack of knowledge about its impact on welfare. This study investigates the effects of chronic nitrate induced stress on *L. vannamei*, adopting a comprehensive approach that includes growth performance, physiological responses, and overall health.

**Table 1.** Survival rates, growth performance indices, and physiological values (Mean +/- SD) for treatments for LN and HN.

| Parameter | LN | HN |
|---|---|---|
| Survival (%) | 98.75 ± 2.5 | 95 ± 4.30 |
| Initial Weight (g) | 15.68 ± 3.15 | 15.68 ± 3.15 |
| Final Weight (g) | 20.17 ± 4.26 | 19.29 ± 4.19 |
| DWG (g/day) | 0.16 ± 0.06[a] | 0.13 ± 0.02[b] |
| SGR (%) | 0.86 ± 0.12[a] | 0.65 ± 0.03[b] |
| Total biomass (g) | 398.38 ± 14.44[a] | 375.15 ± 7.06[b] |
| FCR | 2.39 ± 0.42[a] | 3.17 ± 0.32[b] |
| CF | 0.64 ± 0.02 | 0.65 ± 0.03 |
| HSI | 3.81 ± 0.35 | 3.56 ± 0.61 |
| Glucose (mg/mL) | 39.75 ± 8.35 | 37.5 ± 11.30 |
| Total proteins (g/dL) | 15.16 ± 1.66 | 15.6 ± 2.28 |
| Lactate (g/L) | 0.057 ± 0.038 | 0.057 ± 0.042 |
| Hemocyanin (g/L) | 122.91 ± 31.54 | 114.41 ± 36.33 |
| Hemocyte count ($10^6$ cells/mL) | 15.25 ± 6.83 | 17.21 ± 9.25 |
| Superoxide dismutase (% inhibition rate) | 93.01 ± 5.00 | 94.13 ± 6.52 |

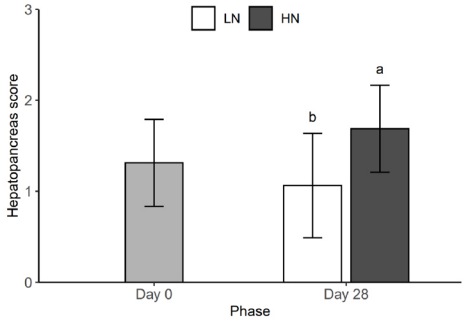


**Figure 1.** Mean value and standard deviation (n = 24) for shrimp hepatopancreas.

## Materials and methods

The experiment was conducted in 8 separate stand-alone tanks filled with 600 L of saltwater (20 ppt). An airlift-operated biofilter with 30L bio-carriers was installed in each tank to establish biofiltration, water movement and gas exchange. Nitrate concentrations were adjusted to two treatment concentration, low: 50 mg/L (LN) and high: 400 mg/L (HN) $NO_3$-N, using sodium nitrate (Carl Roth GmbH, Germany) with four replicates per treatment, for a duration of 4 weeks. Nitrate was measured using a nitrate analyzer probe (subCtech GmbH, Kiel, Germany) and adjusted twice a week to the nominal concentration. Shrimp were counted and weighed for the calculation of biometric indices and growth performance, including survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), and hepatosomatic index (HSI). Hemolymph samples were analysed for glucose and protein in a Fuji DRI-Chem NX500i (Fujifilm Europe) blood analysis system. Plasma lactate levels were quantified with an enzymatic kit (L-lactate, Megazyme). Hemocyte count was done using a light microscope and Neubauer chamber. Hemocyanin was measured following the methods of Chen and Cheng (1993). Hepatopancreas samples were diluted, homogenized in a phosphate buffer and superoxide dismutase (SOD) activity was measured with a SOD determination kit (no. 19160, Sigma-Aldrich, St. Louis, MO). Health parameters were assessed using a non-invasive scoring system for different body areas, originated from literature with slight modifications. Independent sample t-tests, Wilcoxon test, Welch's T-test, Mood median test were used to analyse the difference between the 2 treatments according to the assumptions.

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**Results and Discussion**

No difference was detected in the survival rate between treatments. Variations with other studies may be attributed to differences in salinity, exposure duration, and size which are key factors influencing chronic nitrate toxicity in *L. vannamei* [3]. However, HN impacted the growth performance (SGR, biomass, DWG) and FCR. The growth performance was in line with data reported from this species. As such, reduced feed utilization related to chronic nitrate stress suggests that energy is shifted from growth related processes towards restoration of homeostasis and acclimatization to the altered environment. No significant differences were observed in the physiological parameters analyzed in the hemolymph (glucose, lactate, total proteins, hemocyanin, and hemocyte count) and in the hepatopancreas (SOD activity). These values were comparable to those from unstressed shrimps in other studies. The health index revealed no significant differences, with only the hepatopancreas quality mildly impaired between LN ($1.06 \pm 0.62$) and HN ($1.69 \pm 0.79$). The visual observation of hepatopancreas quality was associated with a change in color from dark brown to pale and a shift in tecture from firm to mushy.

# INTEGRATED NUMERICAL AND FIELD ANALYSIS OF WATER ENVIRONMENT CONTROL INSIDE A MARINE SEMI-CLOSED RECIRCULATING FLEXIBLE FISH CAGE

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## Introduction

The offshore net cage aquaculture industry is increasingly challenged by environmental stressors such as red tides, parasites, and elevated surface water temperatures (Price et al., 2015; Sun et al., 2024). Semi-closed recirculating fish cages are gaining attention as a promising solution to mitigate these issues by physically isolating the cultured environment from surrounding seawater and introducing cooler water from deeper layers. However, understanding and controlling the internal water environment, including water temperature, dissolved oxygen, and salinity—is crucial for improving fish welfare and farming sustainability.

This study focuses on numerical simulation with field experiments to investigate the environmental control performance of a semi-closed recirculating flexible fish cage. The findings aim to provide a scientific basis for optimizing system design and operational strategies.

## Materials and methods

A three-dimensional numerical simulation was conducted by using the MEC (Marine Environmental Committee) Ocean model (Zhang and Kitazawa, 2015), incorporating surface radiative heat exchange and wall heat conduction effects, to investigate the internal temperature distribution of a semi-closed fish cage. The simulation domain is a sub-scale semi-closed fish cage measuring 5 m × 5 m × 3.5 m, with the lower 3 m shaped into a conical structure to facilitate waste accumulation and flow convergence. A structured grid with a uniform resolution of 25 cm was applied both horizontally and vertically.

Water inflow was applied at the top of the cage, while outflow occurred naturally through an open bottom without forced pumping. The temperature of the inflow water was set at 18°C, representing cold water conditions. Seawater properties were assumed with a density of 1025 kg/m³ and a specific heat capacity of 4170 J/kg/K. At the surface boundary, radiative heat exchange was considered. The cage wall boundaries accounted for heat conduction between the internal water body and the external sea water.

Meanwhile, a field experiment was conducted using the same cage configuration in a coastal bay. Cold water (water temperature about 18°C) was pumped from 3 m depth and injected continuously at 250 L/min. Water temperature was monitored at multiple depths.

## Results

Numerical simulation results demonstrate that as the outside seawater temperature increased from 20 °C to 28 °C, the temperature difference between inside and the outside of the cage also increased (Fig. 1). This highlights the system's strong temperature adjust capacity under high-temperature conditions.

Field experiments further validated this performance. In a real coastal expetiment, we compared water temperature inside a semi-closed cage with that of a traditional open-net cage. The semi-closed cage maintained consistently lower temperatures than the open-net cage(Fig. 2). This experimental evidence confirms the system's ability to mitigate heat stress and support a more stable thermal environment for cultured fish.

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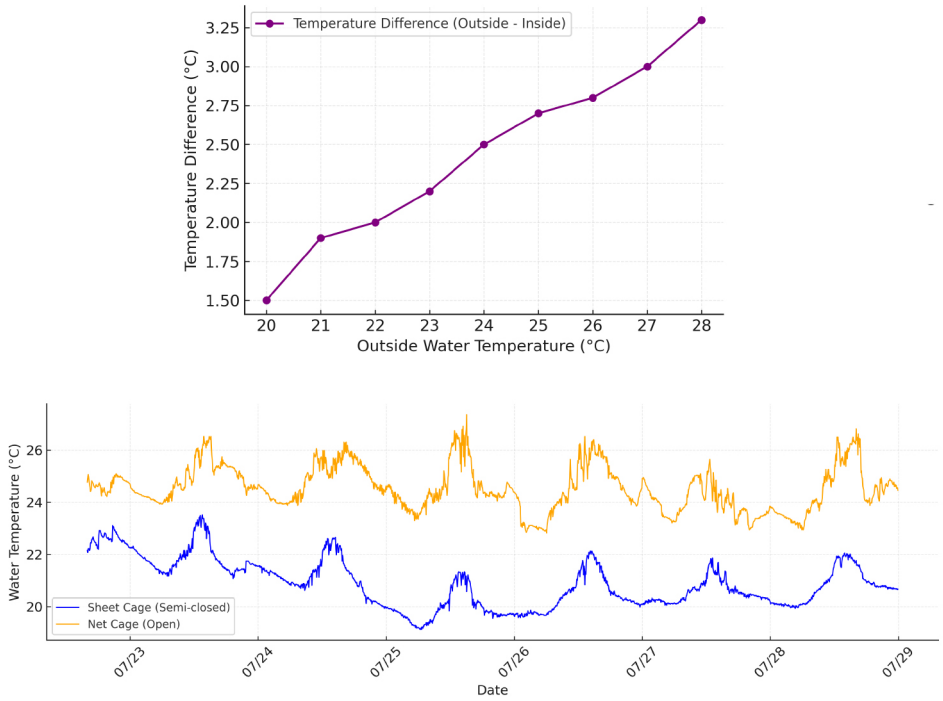


Fig. 2 Water temperature comparison between semi-closed fish cage and open net cage.

## Conclusion

This study evaluated the environmental control performance of a semi-closed fish cage system through both numerical simulations and field experiments. The results revealed that the semi-closed cage effectively maintained a cooler and more stable internal environment. This temperature-buffering capability not only helps mitigate the risks associated with high summer temperatures but also supports a more consistent water environment to fish growth. These findings demonstrate the system's potential for improving environmental stability in marine aquaculture.

## Acknowledgement

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# EFFECTS OF FOOD AVAILABILITY ON THE DYNAMICS OF BIOACCUMULATION AND CLEARANCE OF *Escherichia COLI* IN MUSSELS (*Mytilus galloprovincialis*) AT DIFFERENT TEMPERATURES

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**Introduction**

The coasts of Galicia (NW Spain) are the main production area for bivalves molluscs in the European Union, and mussels (*Mytilus galloprovincilis*) account for about 95% of the production. The Galician coastline is highly populated and industrialized, and huge volumes of wastewater are discharged daily into the rías, which may cause food safety problems due to the consumption of bivalves (Braga et al., 2023; Olalemi et al., 2016).

Official control programs in production areas include the determination of *Escherichia coli* in bivalves. However, monitoring is widely spaced in time (1-2 samplings per month), so that fecal contamination events are usually far from early detection. This would require high-frequency monitoring, but this approach is not possible by sampling of bivalves. However, it might be feasible if water column sample were taken instead, provided that the *E. coli* load in the water could be linked to that bioaccumulated by bivalve molluscs.

The bioaccumulation of *E. coli* in mussels and its subsequent clearance depend on both the concentration of *E. coli* in the water and the time of exposure, but a number of environmental factors can also condition the dynamics of these processes (Braga et al., 2023; Oliveira et al., 2011). The Galician rías are noticeably affected by the northern boundary of the Canary upwelling system (Alvarez et al., 2005), so that mussels are exposed to significant seasonal variations in factors such as food availability and temperature throughout the year, and under these different environmental conditions may be subject to faecal contamination.

Therefore, a pilot-sales study was performed to study the effects of temperature and food availability on the dynamics of *E. coli* accumulation and elimination in mussels, as well as their relationship with physiological, metabolic and behavioural responses.

**Methods**

Non-depurated mussels (*Mytilus galloprovincilis)* farmed in rafts in the Ría de Arousa were placed in a number of 25 L polypropylene tanks. Seawater was supplied in an open-flow circuit (300 mL/min) with a secondary constant supply of seston (10 mL/min) throughout all the experiments. After a 10-day acclimation period, mussels were additionally exposed to a continuous supply of 1000 CFU/ 100 mL of *E. coli* during 48 h in the same flow of seston. Next, the dosage of *E. coli* was stopped and a 48 h-clearance phase was started.

The uptake of *E. coli* by mussels was determined at different periods under different conditions of food availability and temperature found in the Galician Rias. Two consecutive experiments were carried out for that, one at 15 ºC with two simultaneous diets (optimal and suboptimal), and the other with water at 22 ºC and four simultaneous diets (two optimal and two suboptimal). The dynamics of bioaccumulation and depuration of *E. coli* in mussels were defined by monitoring the concentrations of the microorganism in mussels and water throughout the experimental period. Additionally, the filtration and respiration rates of mussels were measured on a daily basis.

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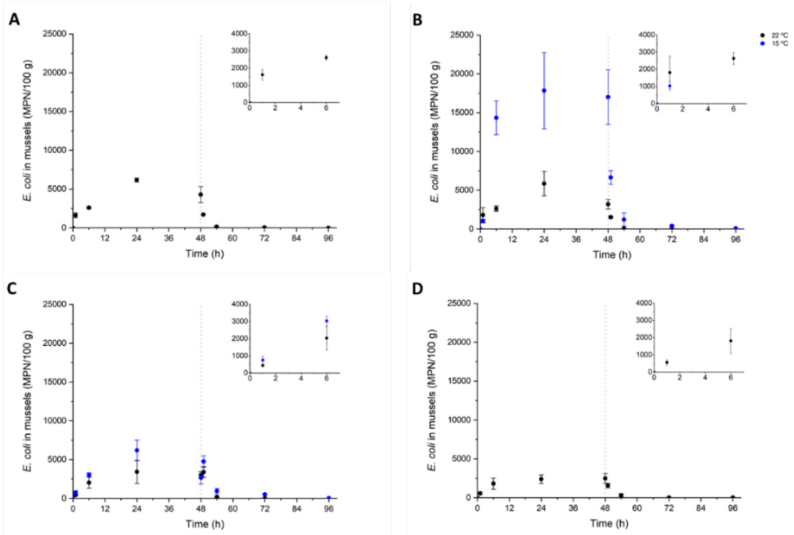


Figure 3. Bioaccumulation and clearance of *E. coli* by mussels as a function of diet and temperature. *E. coli* load in mussels (MPN/100g) fed seston a the following levels: (A) 40.000 cells/mL; (B) 20.000 cells/mL; (C) 2.000 cells/mL; (D) no exogenous input. Data points represent means ± SE (n = 3). Dotted lines at 48 h indicate the end of exposure and the beginning of clearance, The upper window in each graph is the zoom of the first 6 hours of the experiment.

**Results and discussion**

The bioaccumulation of *E. coli* in mussels is a dynamic process in which the bacterial load increases drastically immediately after exposure, reaching concentrations far above those present in the water.

Food availability as well as water temperature and time of exposure were found to have a significant effect on *E.coli* uptake. In the first set of experiments at 15°C, bioaccumulation patterns were similar between treatments, but was observed to reach significantly higher *E.coli* loads in mussels fed the optimal diet from 6 h of exposure to contaminated water. The same way, bioaccumulation profiles obtained at 22 °C showed higher loads of *E. coli* in mussels fed optimal diets than in those fed sub-optimal diets. However, the differences between temperatures were only observed for mussels fed optimal diets, with higher loads of *E. coli* for mussels kept at 15 ºC.

The highest bioaccumulation was observed for mussels with higher filtration activity, corresponding to the optimal diet, especially at 15°C. Seasonal variations in food availability and temperature have a direct effect on the filtration rate and, therefore, to the efficiency of accumulation and purification of microbiological contaminants present in the water column. On the other hand, the higher respiration rates were observed under optimal diets and high temperature, in accordance with the seasonal character of the metabolic activity and the ectothermic condition of the bivalves.

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# EVALUATION OF FAT CONTENT IN FARMED AND WILD SEA BREAM (*Sparus Aurata* L.) THROUGH NON-INVASIVE AND INVASIVE METHODS

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**Introduction**

The gilthead seabream is one of the most valuable species in the Mediterranean basin both for fisheries and aquaculture.

Flesh quality traits, such as fillet fat content and its fatty acids are very important traits for the consumer from a nutritional point of view and the benefits for health. Several studies have confirmed that these parameters are different in farmed and wild fish (Alasalvar et al. 2002).The aim of this study was to determine the fat content in wild and farmed sea bream fillets using different methods (non-invasive vs. invasive) in order to propose a reliable measurement method for use in aquaculture production and breeding programs.

# MODULATION OF THE DIFFERENTIAL PROTEOME OF GILTHEAD SEABREAM (*Sparus aurata*) BRAIN AND SKIN CAUSED BY AN INTRAMUSCULAR INJECTION OF THE HOST DEFENCE PEPTIDE HEPCIDIN 1 (HAMP1)

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Population growth and the declining efficiency of traditional fisheries necessitate an increase in both the production and diversity of cultured species. This drives the application of multiomic molecular techniques to study the immune response of fish, with proteomics being one such technique. Proteomics offers a precise and comprehensive view of the proteome and the changes it may undergo due to intrinsic or extrinsic factors affecting the animal. In this context, we have examined the impact of hepcidin 1 (Hamp1) on the proteome of the brain and skin of sea bream (*Sparus aurata*), a significant species for marine aquaculture in temperate and warm waters [1]. Hamp1 is a host defense peptide (HDP) [2]. HDPs are small, mostly cationic peptides with potent biocidal and immunomodulatory effects. Studying them could enhance fish health in aquaculture, thereby improving fish production and quality. Consequently, in this study, we intramuscularly injected synthetic Hamp1 peptide (50 μL at 25 μM in sterile phosphate buffer). Brain and skin samples were collected for protein extraction using RIPA Buffer and trypsin digestion at 4 and 10 days post-injection. The proteome in the samples was then analyzed by HPLC-MS/MS. Data analysis with Spectrum Mill MS Proteomics software revealed that Hamp1 injection induced changes in the sea bream proteome, as observed in the PCA and PLS-DA, in the brain at 4 days post-injection and in the skin at both 4 and 10 days post-injection.

Among the proteins whose expression varied most in the brain of seabream, sampled four days after peptide injection, were helicase-like transcription factor (HLTF), myelin basic protein (LOC115578167), and α-2-HS-glycoprotein 2 (A0A671UQD9). Additionally, ten days post-injection, RYR3 and α-1,6-mannosyl-glycoprotein 6-β-N-acetylglucosaminyltransferase (MGAT5B) showed increased levels in this organ. Curiously, in the brain, Hamp1 elevated the concentration of proteins within the nucleus, such as HLTF, which plays a role in DNA repair, ATP binding, or helicase/hydrolase activity, as well as proteins in the myelin membrane that serve a structural function.

In contrast, the skin exhibited a greater increase in protein expression compared to the brain. Notably, protein disulfide isomerase (A0A671Y1J8, which is involved in the endoplasmic reticulum stress response), Galectin (LOC115574816), and Alsin Rho guanine guanine (LOC115574816) were elevated four days after injection. Furthermore, Alsin Rho guanine nucleotide exchange factor ALS2 b (ALS2, which is found in the centrosome and plays an active role in cell division) and Peptidyl-prolyl cis-trans isomerase (Ppib, which is associated with RNA polymerase II, protein folding, and isomerase activity) increased at four days, pression of proteins located in the lumen of the endoplasmic reticulum. The protein Galectin, which binds to carbohydrates, galactosides, and laminin and is present in the extracellular matrix or collagen-containing protein, also showed increased expression. Finally, in the skin, we detected an enhanced response to Hamp1 at 10 days post-injection, resulting in a significant increase in proteins while α-1,4 glucan phosphorylase (A0A671V311), Phosphoglycerate kinase (A0A671UGW5), and Rabphilin 3A homolog (mouse), b (RPH3A), among others. A0A671V311 and A0A671UGW5 are present in the cytoplasm and involved in the cell's energy metabolism, including carbohydrate storage, glycogen catabolic process, carbohydrate degradation, glycolysis, pyruvate metabolism, and gluconeogenesis, among others. Furthermore, the expression of RPH3, a protein capable of binding metal ions, GTPase, and involved in intracellular protein transport, exocytosis, and spontaneous neurotransmitter secretion, was also greatly increased. This protein is present in the synaptic vesicle membrane and neurons.

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The findings indicate that the impact of each host defense peptide (HDP) is contingent upon the specific organ under investigation. Furthermore, the results suggest the involvement of certain proteins, either directly or indirectly, in the immunomodulatory effects of HDPs, which may differ depending on the organ. To the best of our knowledge, this study presents the inaugural findings on the effects of HDPs in the brain of a commercially significant fish species. The administration of Hamp1 induces alterations in the proteome of the seabream brain, with more pronounced changes observed at 4 days compared to 10 days. Notably, many of the upregulated proteins are localized within the cell nucleus and are associated with DNA. Conversely, in the skin, the effects of Hamp1 are evident at both 4 and 10 days, with a more substantial proteomic alteration occurring at 10 days. This is characterized by the overexpression of proteins within the endoplasmic reticulum and cytoplasm, facilitating processes such as cell division and neuronal signaling.

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# EFFECT OF ANAESTHETICS ON FISH IMMUNE SYSTEM AND STRESS STATUS

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**Introduction**

Welfare of farmed fish is crucial for production efficiency and economic benefits of aquaculture industry (Jerez-Cepa et al., 2019). However, procedures such as netting, weighing, sorting, vaccination, blood sampling or transport, which are routine practices in aquaculture, can produce stress in fish and compromise their welfare. In this context, anaesthetic agents are widely used during routine practices in order to reduce stress response and improve fish welfare, decreasing physiological damage and susceptibility to pathogens and infections (Martins et al., 2019). Nevertheless, anaesthetic agents may activate and induce by themselves a stress response in fish and compromise their health and welfare (Martins et al., 2019; Soltanian et al., 2018). For this reason and due the few studies where the physiological, immunological and pathological effects of anaesthetic agents have been analyzed in fish, in this work we analyzed the effect of exposition to three anaesthetics, clove oil (CO), tricaine methanesulfonate (MS-222) and chlorobutanol (CB) on immune, stress and antioxidant status of gilthead seabream (*Sparus aurata*) and greater amberjack (*Seriola dumerili*) specimens.

**Material and methods**

Gilthead seabream and greater amberjack specimens were completely anesthetized with different anaesthetic agents and recovered in water without anaesthetics and plenty of oxygenation. Blood samples were taken at 0, 1 and 24 hours after recovery. For that fish were slaughtered by anaesthetic overdose. Water samples were taken at 0, 5, 30 and 60 min. The control group was only slaughtered in a similar manner. Immune response and antioxidant activities, as well as stress status, were measured according to previous studies (García-Beltrán et al., 2024).

**Results**

Regarding results in gilthead seabream, CO increased serum protease activity at 0 and 1 hours while decreased lactate level at every sampled time. For its part, MS-222 increased serum cortisol level at every sampled time, as well as glucose and lactate levels at 1 hour.

As to greater amberjack, similarly to gilthead seabream, CO decreased serum glucose and lactate level at 24 hours as well as cortisol level in water at 1 hour. Meanwhile, MS-222 decreased serum bactericidal activity at 0 hours while increased cortisol and lactate levels at 0 and 1 hours and decreased them at 24 hours. Finally, CB increased serum cortisol level at every sampled time and decreased lactate level at 1 and 24 hours, as well as total antioxidant activity at every sampled time.

**Discussion**

Anaesthesia, which includes partial or total loss of sensation, voluntary neuromotor control, mobility, equilibrium and consciousness through depression of the central and peripheral nervous system (Zahl et al., 2012; Martins et al., 2019) is a common practice in fish aquaculture during handling routine procedures in order to reduce fish stress response and susceptibility to pathogens and infections. However, fish response to anaesthetic agents is species-specific and dependent on compounds chemical properties (Jerez-Cepa et al., 2019). Thus, anaesthetic agents may activate and induce by themselves a stress response in fish and compromise their health and welfare (Martins et al., 2019) and induce negative effect on growth, physiology, haematology and biochemistry of fish and appearance of disease outbreaks which can lead to mortality (Soltanian et al., 2018). Therefore, in order to obtain the best efficacy of anaesthesia avoiding negative effects on fish it is necessary to select the correct anaesthetic agent, dose and duration, which vary between and within species (Zahl et al., 2012; Martins et al., 2019).

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In this work, we study the effect of anaesthetic agents on immune activities, on fish stress status and on antioxidant activity of fish. Considering, all results, we can conclude that MS-222 and CB caused a short acute stress situation in fish which returned to normal level after a few hours and did not negatively affect to fish immune system. However, exposition of greater amberjack to CB negatively affected antioxidant status. For its part, any negative effect was observed in immune, antioxidant or stress status in fish exposed to CO. Therefore, it can be concluded that CO and MS-222 can be considered safe for gilthead seabream and greater amberjack specimens and precautions should be taken when using CB.

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# MICROALGAE AND ANTIMICROBIAL PEPTIDES (AMPs) FROM MICROALGAE AS POTENTIAL TOOLS FOR FISH AQUACULTURE

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**Introduction**

Microalgae represent a natural and sustainable alternative to the use of antibiotics in aquaculture, both due to their nutritional characteristics and their potential as immunostimulants (Charoomart et al., 2018). These microalgae have the ability to combat pathogens, diseases and stress due to the large number of bioactive compounds they present, including antimicrobial peptides (AMPs) which play a crucial role in the immune system of living organisms as the first line of defence against a wide variety of pathogens. These AMPs can directly lyse microorganisms, act as regulators of the immune system in pathogen elimination and inflammation and exhibit antioxidant activity (Zhang et al., 2022). Therefore, in this study, the antioxidant, antibacterial and immunostimulatory activities of microalgae treated or not (with or without cell wall disruption), and AMPs from microalgae were analysed.

**Material and methods**

Microalgae (*Chlorella vulgaris*, *Dunaliella salina*, *Haematococcus pluvialis*, *Nannochloropsis gaditana*, *Tisochrysis lutea* and *Schizochytrium* sp.), macroalga *Alaria esculenta* and cyanobacteria *Arthrospira platensis* cells were harvested and lysed by enzymatic hydrolysis to release soluble cytoplasmic proteins (Galafat et al., 2020). Controls were included by incubating cells in the hydrolysis buffer in the absence of enzymes. After centrifugation, the soluble fraction and the cellular fraction were obtained, the latter being homogenized. Moreover, five AMPs from microalgae were selected and synthetized for *in vitro* studies (AP1, AP2 and NV14 from *A. platensis*, APWP from *C. vulgaris* and DS1 from *D. salina*).

Antioxidant, antibacterial and immunostimulant activities of microalgae and AMPs were studied *in vitro* as previous studies (García Beltrán et al. 2018). The capacity of samples to reduce the radical cation $ABTS^{+}$ was used to determine antioxidant activity. Antibacterial activity was tested by measuring the inhibition of growth of three fish-pathogenic bacteria: *Vibrio anguillarum* (Va), *Vibrio harveyi* (Vh) and *Photobacterium damselae* subp. *piscicida* (Phb). Finally, the ability to stimulate peroxidase (formation of bactericidal hypochlorous acid) and respiratory burst (activation and production of reactive oxygen species, ROS) activities by head-kidney (HK) leucocytes of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) was studied.

**Results**

Microalgae *D. salina*, *T. lutea* and macroalgae *A. esculenta* showed the highest antioxidant activity. As to antibacterial activity, *D. salina*, *T. lutea* and *Schizochytrium* sp. showed the best activity as well as *H. pluvialis* and *N. gaditana* against Vh and Va and Phb respectively. For its part, *C. vulgaris*, *H. pluvialis* and *A. platensis* showed the highest activity against Vh. Interestingly, all AMPs showed antioxidant and antibacterial activity, except DS1 which did not show antibacterial activity against any bacteria. AP1 and APWP showed the best antioxidant activity, while AP1 and AP2 showed the best antibacterial activity. However, NV14 and APWP did not show activity against Phb, and Vh respectively, but did against Va.

As to immunostimulant activity, cellular fractions of all microalgae as well as supernatant fractions of *A. platensis* and *A. esculenta* increased peroxidase activity of gilthead seabream and European seabass HK leucocytes. Concerning respiratory burst activity, in the case of gilthead seabream HK leucocytes, all supernatant fractions of microalgae increased values while most of cellular fractions decreased this parameter. For European seabass HK leucocytes, cellular fraction of *Schizochytrium* sp. increased it while cellular fractions of *C. vulgaris*, *N. gaditana* and *A. platensis* and supernatant fraction of *A. esculenta*

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showed the opposite effect. Finally, all AMPs increased the peroxidase activity in both gilthead seabream and European seabass HK leucocytes. Moreover, AP1, AP2 and NV14 increased the respiratory burst activity of both gilthead seabream and European seabass HK leucocytes, while APWP and DS1 decreased values in gilthead seabream cells and had no effect on European seabass HK leucocytes.

**Discussion**

Microalgae have great potential as a source of bioactive molecules capable of regulating immune, antioxidant and antibacterial responses. However, research about these activities directly from microalgae or through the release of their bioactive compounds is needed (Charoonnart et al., 2018).

Regarding antioxidant and antibacterial activity, enzymatic hydrolysis generally increased the activities of both fractions studied compared to the data obtained for untreated biomass. Moreover, supernatant fractions of microalgae showed higher activity than cellular fractions, especially in the case of antioxidant activity. Therefore, results indicate that both microalgal biomass processing method and fractioning affect bioactive compounds activity, thus explaining the wide variability of effects described in the literature (Charoonnart et al., 2018). AMPs also exhibited both activities, especially AP1, AP2, and APWP. As to immunostimulatory activity, both microalgae and AMPs increased the peroxidase activity of gilthead seabream and European seabass HK leucocytes. However, increased and decreased respiratory burst activity observed could be due to the antioxidant activity of both microalgae and AMPs, which are able to stimulate HK leucocytes and, at the same time, eliminate ROS created by this activation. Therefore, both microalgae and AMPs are showed as potential tools for use in aquaculture.

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# TRANSCRIPTOMICS AND ITS DEPENDANCE ON THE GENETIC FRAMEWORK OF GILTHEAD SEABREAM

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## Introduction

Improved growth and disease resistance are crucial in aquaculture production (Houston et al., 2020). During their life cycle, fish release their eggs into the environment, and after fertilization, they hatch in an environment with pathogens and with their immune system in the early stages of development (Mulero et al., 2007). Therefore, there is a relationship between and effective immune system of embryos and their survival rate (Mulero et al., 2007; Cordero et al., 2016). However, there is little information on the ontogeny of the immune system in gilthead seabream (*Sparus aurata*) and nothing is known about how these ontogenetic processes may be affected by growth-based genetic selection (Cordero et al., 2016). For this reason, in this work we performed a miRNA transcriptomic analysis of gilthead seabream eggs and larvae from two different populations of breeders differentially selected for growth from the genetic selection program of PROGENSA and Centro Oceanográfico de Murcia (COMU-IEO), CSIC.

## Material and methods

Two distinct groups (A1 and A2) of gilthead seabream breeding stock from different sources were used. One was from the PROGENSA breeding program and maintained at the COMU-IEO, CSIC facilities (A1, specimens of 4-5 years old), and the other was from the Mediterranean Sea and maintained at the COMU-IEO, CSIC facilities (A2, specimens of around 15 years old).

Eggs from three different clutches and 66-day-old larvae of A1 or A2 spawns were sampled for transcriptome analysis (García-Beltrán et al., 2024; Kanehisa et al., 2025).

## Results

Significantly greater differences in expression patterns (KEGG pathways) were observed in larvae than in eggs from different breeders.

The main differences in gene expression patterns in eggs are related to metabolism of lipid (glycerophospholipid), fatty acids (linoleic acid, α-linolenic acid and arachidonic acid), amino acids (arginine, proline, glycine, serine and threonine) and nucleic acid (pyrimidine), immune system (herpes simplex virus 1 infection, intestinal immune network for IgA production and phagosome), signalling pathways (MAPK, calcium and adrenergic signalling pathways), apoptosis, cellular and focal adhesion molecules, and ligand-receptor interactions (ECM-receptor and neuroactive ligand-receptor interaction). Meanwhile, the main differences in expression patterns in larvae from different breeders are related to important signalling pathways involved in energy homeostasis and cell metabolism, development, apoptosis, migration and proliferation (PPAR, adipocytokine, mTOR, VEFG, wnt and GnRH) and immune response regulation (PPAR, erbB and c-type lectin receptor). Moreover, differences in expression of genes related with endocytosis, cellular and focal adhesion molecules (tight junction, adherents' junction and focal adhesion), energy metabolism (oxidative phosphorylation), lipid metabolism (glycerophospholipid), general metabolism (glyoxylate and dicarboxylate) and xenobiotics and drugs metabolism (cytochrome P450) were also observed.

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## Discussion

The study of fish ontogeny is a key knowledge to understand how embryos develop and which genes related to survival are expressed from the moment of eggs fertilization (Houston et al., 2020). The embryos of fish, when released into a hostile environment, have to provide defense mechanisms that allow them to survive and grow as quickly as possible (Mulero et al., 2007). A quick growth is also important for aquaculture sector purposes. As gilthead seabream (*Sparus aurata*) is one of the most cultivated species in
Spain and in the European Union, genetic selection for growth is being established (Vallecillos et al., 2021).

In this work, eggs from different breeders showed more significant differences in the expression patterns of a smaller number of genes than larvae, which showed less significant differences but in a larger number of genes. Therefore, results obtained in this work revealed that studying the ontogeny of gilthead seabream is key to understanding the development of the species and individual survival, identifying key genes at each stage of the process.

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# ANTIVIRAL POTENTIAL OF PLANT EXTRACTS AGAINST RED SPOTTED GROUPER NERVOUS NECROSIS VIRUS (RGNNV)

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Infectious diseases pose a significant challenge to aquaculture, resulting in significant economic losses. Treatment options for viral infections in particular are limited. The use of chemotherapeutic agents has several drawbacks, including adverse health and environmental effects. Therefore, there is an urgent need for new antiviral or therapeutic agents that are effective, affordable and sustainable [1]. This study proposes the use of plant extracts as sources of antivirals that not only contribute to more sustainable aquaculture practices but also utilise by-products from terrestrial industries such as agriculture for the development of these treatments.

This study focuses on RGNNV, the most widespread strain affecting numerous fish species, with European seabass being highly susceptible. RGNNV causes up to 100% mortality in larvae and juveniles [2] and significant losses in adult fish by targeting central nervous tissue, particularly the retina and brain, resulting in extensive vacuolization and neuronal degeneration. Symptoms include erratic swimming, loss of appetite and hyperinflation of the swim bladder [3].

In this study, we evaluated the in vivo antiviral activity and immunomodulatory capacity of five plant extracts encapsulated and formulated in the feed. First, we assessed the health status of the individuals fed with the supplemented feeds, for 3, 10 and 28 days. We evaluated parameters such as growth, blood biochemistry, haematology and gene expression in various tissues including anterior kidney, intestine and erythrocytes. Results showed a slight increase in growth parameters in the groups fed with the feed supplemented with the plant extracts in comparison with the control. However, blood biochemistry and haematology profiles results varied depending on the treatment and the days on which it was analysed. In addition, the antiviral capacity of the feeds supplemented with the plant extracts was assessed *in vivo* by means of a challenge with RGNNV. Results showed that some plant extracts increased the survival of the fish by up to 20%. Regarding immunoglobulin titration, we observed an increment in the production of specific IgMs against RGNNV in the case of some plant extracts.

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# DECODING BIOLOGICAL AGEING TO ENHANCE WELFARE IN FARMED SEA BREAM: A TRANSCRIPTIONAL AND EPIGENETIC APPROACH

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## Introduction

As aquaculture expands to meet global food demands, the need for innovative tools to monitor fish welfare and wellbeing is growing. Biological age (BA), unlike chronological age, offers a more accurate measure of overall welfare by reflecting the cumulative effects of internal and external stressors. Consequently, BA markers can serve as highly informative welfare indicators since their activity is highly influenced by the environment (Colditz *et al*., 2023). In a previous multi-omics study in farmed gilthead sea bream, we applied an integrative transcriptomic and epigenetic approach to target BA in skeletal muscle, focusing on genes with an opposite trend of DNA methylation and expression (Belenguer *et al*., 2024). From an initial list of 102 genes, 10 top candidate markers were retrieved based on their multifunctionality and differential methylation in regulatory regions. As a result of this, the up-regulation of *sirt1*, *smad1*, *ramp1* and *psmd2,* in combination with the down-regulation of the other six genes *(col5a1*, *calcrl*, *bmp1*, *thrb*, *spred2*, *atp1a2)* was related to an older phenotype. The present study aims to validate these selected markers of BA under varying independent aquaculture conditions, evaluating their potential utility as generic molecular indicators of fish welfare.

## Materials and methods

Four independent trials were conducted to assess how different environmental and nutritional challenges modulate the gene expression patterns of BA selected candidate markers. In the first trial (HD), 2-year-old fish (~480 g) were stocked at two different densities and water [$O_2$] levels: control (8.5 kg/m$^3$; 5–6 ppm) and high density (HD, 25 kg/m$^3$; 3–4 ppm) for 8 weeks (Holhorea *et al.*, 2023). In the remaining trials, 9–12-month-old fish (50–105 g) were fed different experimental diets to evaluate their potential benefits under either thermal stress (TS) or standard conditions. In the TS trial, the experimental diet was supplemented with a hydrophilic emulsifier administered during the extreme heat episode in the summer of 2022 (Domingo-Bretón *et al*., 2025). In the other trials, the potential benefits of a porcine protein hydrolysate enriched in bioactive peptides (PHS trial, 5% inclusion level), and microalgae biomass (*Nannochloropsis oceanica*) derived from the valorisation of brewery waste (NCHL trial, 14% inclusion level) were assessed over 10–14 weeks in fast-growing fish. RNA was extracted from white skeletal muscle (500 ng) and reverse transcribed prior to gene expression analysis by RT-qPCR. All pipetting operations were carried out using an EpMotion 5070 Liquid Handling Robot. β-actin was used as the housekeeping gene, and multigene expression data were normalised to *smad1* expression in control fish of each trial, which was arbitrarily set to 1. Gene expression differences ($p < 0.05$) were assessed using a non-parametric Kruskal–Wallis test followed by Holm–Sidak *post hoc* analysis. Clustering of gene fold changes was performed using complete linkage. Biological process categories from Gene Ontology (GO-BP) associated with each gene were retrieved from Belenguer *et al*. (2024).

## Results and discussion

As shown in Figure 1, all 10 candidate genes responded to at least one of the four experimental conditions, validating their sensitivity to both environmental and nutritional challenges. In the HD trial, six genes were either up-regulated or down-regulated, reflecting an older phenotype associated with impaired welfare and wellbeing due to the stressful condition. Conversely, in the other trials, the observed changes, 5 up- and 2 down-regulated genes, revealed a younger phenotype, likely resulting from improved performance following dietary supplementation with a hydrophobic emulsifier (TS trial), protein hydrolysate (PHS trial) or microalgae biomass derived from the brewery by-product valorisation (NCHL trial). When clustering the gene expression fold-changes, the trials grouped according to the direction and magnitude of their transcriptional responses. Trials yielding a younger phenotype (TS, PHS, and NCHL) clustered together, while HD emerged as an outlier due to its distinct stress-related expression profile, indicative of an older phenotype. Functionally, the tested genes were associated with

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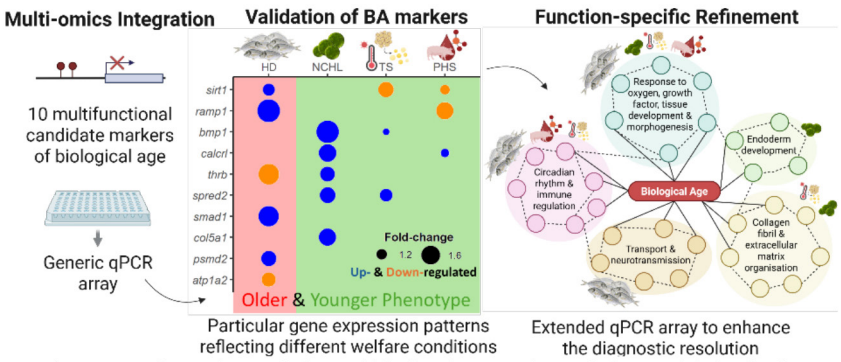


**Figure 1.** Overview of the validation/annotation of the 10 biological age candidate markers in gilthead sea bream.

71 GO-BP terms, which were categorised into challenge-specific and shared responses. Challenge-specific terms included transport and neurotransmission in the HD trial, and endoderm development in the NCHL trial. Shared functions across all four trials included tissue development (especially vascular development), morphogenesis, growth factor signalling, and responses to oxygen-containing compounds. Additional overlaps were observed: HD, TS, and NCHL were linked to sensory and skeletal development; HD, PHS, and NCHL to cell signalling and heart development; HD, PHS, and TS to circadian rhythm and immune regulation; TS and NCHL to collagen fibril and extracellular matrix organization; HD and NCHL to early embryonic development. These biological functions are tightly regulated during development but become progressively dysregulated with ageing under stressful conditions due to cumulative molecular damage, altered signalling, and impaired tissue maintenance and repair (La Mesa and Eastman, 2024). The sensitivity of these genes to environmental stressors further underscores their value as markers for tracking BA beyond chronological age.

## Conclusions

This study confirms the potential of a set of 10 candidate genes as versatile biomarkers of BA in gilthead sea bream. By incorporating function-specific refinements, the validated qPCR array can be further customised with challenge-responsive markers, significantly boosting its diagnostic power. This innovative approach paves the way for a reliable and forward-looking tool to support the certification and auditing of animal welfare and wellbeing in aquaculture systems.

## Funding

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# INFLUENCE OF THE HYPOOSMOTIC CONDITIONS ON INTRACELLULAR DIGESTION OF THE EUROPEAN FLAT OYSTER (*Ostrea edulis*) BROODSTOCK

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## Introduction

Digestion in shellfish occurs extracellularly and intracellularly. The main site of intracellular digestion is the digestive cells located in the tubules of the digestive gland. During each digestion process, these cells go through four phases (resting, absorption, degradation, and reconstitution), and the diameter of the lumen of the digestive tubules also changes (Gavrilović, 2011). Determination of the digestive tubule index can indicate a lack of food or unfavorable (stressful) conditions in the culture environment in which filtration is reduced (Winstead, 1995; 1998). Given the rhythmic changes in the digestive cells, sampling should be carried out at shorter time intervals within a day to obtain relevant data. Considering that *Ostrea edulis* is often grown in areas near estuaries and/or underground fresh water sources, the aim of this research was to determine whether reduced salinity (hypoosmotic) affects food ingestion, i.e. the digestion process to optimize the gonad conditioning process in the hatchery.

## Materials and Methods

Immediately before the start of the month-long experiment, two recirculating oyster conditioning systems were set up. Each system was stocked with 200 oysters from a farm in Mali Ston Bay. The basic parameters of the culture medium (oxygen saturation, temperature, salinity and pH) were kept constant and regularly monitored. Salinity in both systems was maintained at 35 psu during the first seven days of adaptation to the new breeding environment. After this period, in the first experimental group it was maintained at the same value, while the second experimental group was gradually acclimatized to 20 psu over the course of seven days. Feeding was carried out by giving an algae mixture in the amount of 3% of the dry mass of the soft oyster tissue. Four species of unicellular algae were used as feed: *Isochrysis galbana, Pavlova lutheri, Tetraselmis suecica* and *Cylindrotheca closterium.* The ratio of the listed algae species in the diet was 20%: 20% : 20% : 40%. The required volume of algae suspension was calculated according to Gavrilović et al. (2021). After one month of the experiment, 30 individuals from each system were sampled five times at one and a half hour intervals (total 150 per system). All oysters were opened and the soft tissue was fixed in Davidson fixative for routine histological processing. Hematoxylin-oezoin was used for staining. After microscopic examination, the digestive tubules index was calculated according to the method described by Winstead (1995). The mean value of the tubule index was calculated for each individual and then the mean value for each experimental group. ANOVA test was used to compare the values of tubule condition index at different sampling times of the same experimental group. After this test, the similarity of the mentioned groups was analyzed using MRT (Multiple range test). A statistical comparison of the values of the digestive tubule index between the group kept at 35 psu and 20 psu salinity was performed using the Chi square test.

## Results and Discussion

The results of a microscopic examination of the digestive gland of both groups have shown that there is a rhythmical exchange of phases in the digestive tubules (Table 1), where in a single oyster the exchange was not synchronous in all of the digestive tubules, but they were in phases that were close to one another. Depending on the sampling interval all four phases of digestive tubules were found: resting, absorption, degradation, and reconstitution. Within the same experimental group ANOVA test has shown a significant statistical difference between individual sampling intervals ($p<0,05$), which confirms the existence of a rhythmical exchange in the digestive rhythm in the tubules. The results of a statistical comparison using a CHI SQUARE test showed that there was no significant statistical difference in the values measured between the two salinity groups ($\chi^2<0.711$, $p<0,05$). Winstead (1995; 1998) found that lower salinity affects intercellular digestion in *Crassostrea virginica*. Horedesky et. al (2019) reported optimal salinity for *Crassostrea gasar* in the range of 4 to 40 psu. From our results it can be concluded that *O. edulis* can gradually adapt to a 20 psu salinity, with no significant changes in the feeding rhythm, digestion rhythm and the digestive tubules index regarding the tested salinity value.

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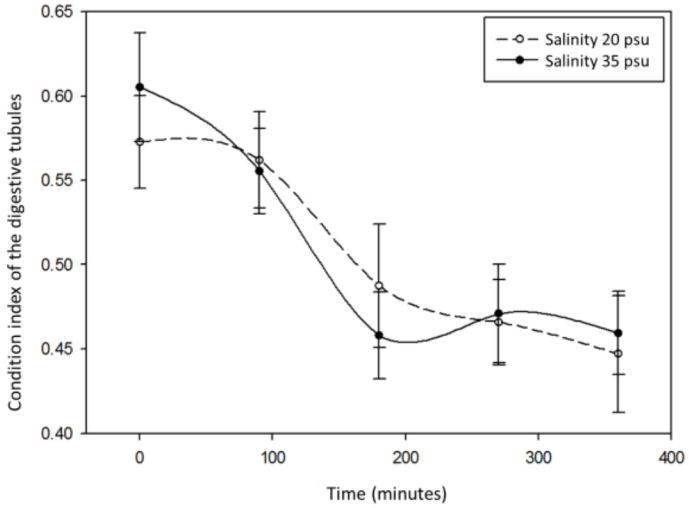


Table 1. Comparison of mean values of the digestive tubule condition indices at salinity of 20 and 35 psu over the sampling period

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# CAN AIR NANOBUBBLES IMPROVE THE SWIM BLADDER INFLATION IN DEVELOPING EUROPEAN PERCH? A PILOT STUDY OF ADVANCED WATER TREATMENT

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## Introduction

Swim bladder inflation (SBI) is a fundamental step in larval development for both physostome and physoclist fish species (Balon, 1975; Price and Mager, 2020). Failure of the SBI triggers higher energy expenditures of the individual, which negatively affects swimming ability, growth rate, feeding efficiency, susceptibility to predation, and survival rate (McElman and Balon, 1979; Summerfelt, 1996). In aquaculture, the SBI failure is caused by different factors including: (a) water contamination with oil increasing the surface water tension and preventing the larvae from gulping air bubbles for inflation, and (b) bacterial aerocystitis of the swim bladder caused by gulped organic debris (Summerfelt, 2013). Hence, novel and effective water treatment technologies such as nanobubbles can be employed in aquaculture to reduce the risk of low SBI. This study aimed to apply the air nanobubbles alone and in combination with conventional water treatment technologies, *i.e.* spray and water skimmers to promote swim bladder inflation success in European perch larvae under aquaculture rearing conditions.

## Materials and methods

Four experimental treatments were conducted in a triplicate design to investigate the effect on SBI of European perch larvae: (a) surface spray covering one-third of the tank diameter and skimmers (SS); (b) air nanobubbles (ANBs); (c) surface spray covering one-third of the tank diameter, skimmers and air nanobubbles (ANBsSS); and (d) control with no water treatment technology. To prevent cross-contamination of NBs between treatments and control groups, the experiment was done in two recirculating aquaculture systems (RASs). Larvae from all four experimental conditions were sampled at the $6^{th}$, $9^{th}$, $12^{th}$, $15^{th}$, $18^{th}$, and $21^{st}$ dph. For each sampling point, 30 individuals per tank (90 ind. per treatment) were killed by a lethal dose of clove oil (Hamackova et al. 2006) and weighed to the nearest 0.10 mg. Measuring the individual standard length (SL), counting larvae with inflated and non-inflated swim bladder and determining the swim bladder length (SBL) were done. Swim bladder inflation efficiency (SBIE) was calculated as the number of larvae with inflated swim bladder/total larvae examined x 100. Survival rate (SR) was determined as the percentage of surviving larvae in relation to the total number of stocked larvae in each sample. Viability was calculated as the SR × SBI /100 = (S × GBI )/100, where SR is the percent survival rate, and SBI is the percentage of the survivors that had an inflated swim bladder (Clayton and Summerfelt, 2010). The yield presents the number of harvested juveniles with inflated swim bladder in the total water volume of the rearing tank (Ljubobratović, et al., 2019). the nanobubble generator was connected to the RAS system with the respective experimental treatments, *i.e.* ANBs and ANBsSS. Water from the retention tank was pumped into the nanobubble generator and then to tanks once per hour for 15 min.

## Results

At the end of the experiment, the final SBL and SL of the larvae were not significantly different ($p \leq 0.05$) across all treatments. The final BW was approximately 1.5-fold higher in all treatment groups compared to the control groups. The low BW of the control groups seems to be in line with the final SBIE that is 2.2- to 2.4-fold higher in SS- and ANBsSS larvae with SBI compared to the control groups. In contrast, the SR was 2-fold higher in individuals from SS and ANBsSS groups compared to the control groups. A very low final SR (19.57%) was also recorded in the ANBs treatment group, although final SBIE and BW were in comparable ranges. The viability and yield were significantly higher in ANBsSS group, followed by SS group, while ANBs and control groups did not differ.

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**Discussion and conclusion**

All treatments showed higher SBI efficiency compared to the control, where no water treatment technology was employed. Higher SR and SBIE and consequebtly higher viability and yield were found in individuals from SS and ANBsSS groups. This may be explained by the beneficial combination of SS and ANBsSS to remove the oil film layer on the water surface. The resulting enhanced SBIE observed in our study, invariably achieved better larvae growth gain, what was also shown for ANBs but with lower SR. A higher SR in the ANBsSS group could be achieved by the Brownian motion of the air NBs, which produce an "up-welling" effect and stirs up small and passively swimming larvae in the water suspension, thus minimizing their swimming requirements(Kolkovski et al.,2004). However, applying ANBs alone resulted in an unexcepted low SR especially in early larval stage. Hence, appling NBs later in the larval stage can result in an additive effect to already used technology such as surface sprays, skimmers and upwelling. Moreover, different types of cannibalism should be also explored in future studies to get an overall picture of the NBs effect.This puzzling observation remains to be investigated in the future because there are still gaps in the effects of ANBs on larvae's SB inflammation processes.The current study offers the first investigation of the nanobubbles' effect on SBI promotion in larvae of European perch and gives motivation for future optimizations.

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# MOBILE LICE DETACH DURING CROWDING BUT CAN BE RETAINED BY FINE-MESHED CROWDING NET

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## Introduction

Crowding is a common practice in salmon aquaculture during operations such as sea lice treatments and harvesting. Sea lice, particularly *Lepeophtheirus salmonis,* remain one of the most significant parasitic challenges in Atlantic salmon farming. A growing concern is that during crowding events, mobile lice detach from their hosts and spread into the surrounding environment where they may pose a reinfestation risk for both farmed and wild fish. Despite these concerns, the extent to which lice detach and are lost during these events is still not well understood. The aim for this project was therefore to quantify lice detachment, determine the mesh size needed to capture all lice stages, and assess the feasibility of using a fine-meshed net to collect detached lice during crowding.

## Methods

Replicate crowding trials were conducted in small research cages, using either a standard coarse-mesh crowding net or a fine-meshed crowding net intended to retain detached lice. The trials varied in crowding duration (10 min – 2 h), fish size and number, as well as louse density. Detached lice that passed through the crowding nets were captured using a plankton net, allowing direct quantification of lice that were 'lost' during the crowding process. Additionally, a benchtop physical model was made simulating the retention of detached lice using various mesh types and sizes (0.8–2.0 mm) as well as precise measurements of each louse stage size metrics.

## Results and discussion

Mobile lice that detached during crowding varied from 2 to 38%, with higher detachment rates during longer crowding duration (up to 2 h) and with larger fish. Smaller pre-adult lice detached more frequently than adult lice. Laboratory studies demonstrated that a nominal mesh size of 0.8 mm effectively captured all lice stages, 1.6 mm retained most pre-adults and adult males, while adult females required a 2.0 mm mesh for successful collection. The maximum mesh opening size (diagonally) corresponded to the cephalothorax width of the louse stage captured. In cage trials, the fine-meshed crowding net successfully retained 75% of adult female lice and improved fish welfare. Overall, these findings show that significant lice loss can occur during handling of salmon and that collecting lice during crowding operations could be an effective management strategy to limit their release into the environment and thereby lower the reinfestation risks for both farmed and wild fish.

# ASSESSING THE POPULATION GENETIC STRUCTURE OF WILD GILTHEAD SEABREAM (*Sparus aurata* Linnaeus, 1758) OVER THE PAST 3 DECADES

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## Introduction

Marine aquaculture has expanded rapidly in Greece and the Mediterranean in recent decades, with Gilthead seabream (*Sparus aurata* Linnaeus, 1758) being one of the two most extensively farmed species (Greece: 66,000 tonnes in 2022; HAPO 2024). As a result, fish escapes from aquaculture facilities may have significant ecological impacts on local wild populations, including disease transmission, competition for resources, and the introgression of foreign genetic material (Arechavala et al. 2018). Gilthead seabream is distributed from the Black Sea and the Mediterranean to the eastern Atlantic. Existing literature shows either an absence or very weak genetic differentiation among populations, with Greek stocks demonstrating genetic homogeneity (Geladakis et al. 2023 and references therein). However, wild populations are typically characterized by higher genetic diversity compared to farmed populations (Geladakis et al. 2023 and references therein). The objective of this study is to investigate the potential genetic effects of aquaculture escapees on the genetic diversity of wild Gilthead seabream populations in Greece by comparing samples from the early aquaculture period (1993–1997, annual production of 5–18 thousand tonnes; Dimitriou et al. 2007) to contemporary populations (2015-2024).

## Material and Methods

Total genomic DNA was isolated from historical scale samples of 86 Gilthead seabream individuals (1993–1997, Messolonghi Lagoon, Western Greece) and from tissue samples of 227 individuals collected between 2015 and 2024 in the Messolonghi Lagoon and the Ionian Sea. Building on the findings of Geladakis et al. (2023), which indicate that the likelihood of aquaculture escapees increases proportionally to the degree of scale regeneration (SRD, % regenerated scales), SRD was used as a criterion to assess potential impacts of escapees on the wild gene pool. Accordingly, wild fish were classified into two predefined categories based on their SRD values: (a) individuals with SRD $\leq$ 30% (L30) and (b) individuals with SRD > 30% (M30). In addition, DNA was isolated from three groups of reared samples serving as reference points: (i) 48 fish from the Ionian Sea collected in 2012, (ii) 27 fish from the Ionian Sea collected in 2016, and (iii) 16 fish from the Northwestern Aegean Sea collected in 2015. All individuals were genotyped for 15 microsatellite loci. Genetic diversity and genetic differentiation among groups ($F_{ST}$ values) were estimated using GenAlEx (v. 6.5) whereas the effective population sizes ($N_e$) were estimated with NeEstimator (v. 1.3). The population structure was inferred through Bayesian clustering analysis using STRUCTURE software (v. 2.3.4).

## Results & Discussion

Genetic analyses revealed no significant differentiation ($F_{ST}$ = 0.003–0.024, p > 0.05; Figure 1B) between historical (1993–1997) and contemporary (2015–2024) Gilthead seabream samples from the Ionian Sea, exhibited long-term stability in the genetic structure of wild populations. Similarly, no genetic differences were detected among wild fish classified by their degree of scale regeneration (SRD), used here as a proxy for the potential presence of aquaculture escapees (p > 0.05; Figure 1B). Interestingly, the $N_e$ remain high, comparable to recent studies of contemporary groups of Gilthead seabream (Žužul et al., 2019). These findings suggest that large effective population sizes, widespread larval dispersal, and adult migratory behavior maintain panmictic population dynamics of large breeding populations, preventing detectable temporal genetic shifts. Consequently, the detrimental interaction of aquaculture to the natural resources of the species cannot be documented.

In contrast, a small but statistically significant differentiation was observed between wild and farmed stocks ($F_{ST}$ = 0.016–0.044, p < 0.05), with wild populations consistently displaying higher levels of genetic variance (Figure 1A, 1B). Reared fish retain only a small subset of the natural genetic diversity, often further diminished by artificial selection and intensive breeding practices (Arachevala et al., 2018. The Ne of the farmed fish is 20 (from the 1995 population) to >400 (from the contemporary populations) times smaller than the wild (Figure 1A). Despite recurrent escape events from offshore aquaculture facilities (Dempster et al., 2018), introgression of farmed genotypes into wild populations appears to be minimal. This likely reflects the large effective population size of wild seabream stocks and/or the reduced survival and reproductive success of escapees under natural conditions.

*(Continued on next page)*

A.

| Fish Group | | n | A | Ae | Ar | Ho | He | Fis | Ne (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| 1995 | L30 | 68 | 12.2 ± 6.84 | 6.62 ± 1.12 | 5.81 ± 1.99 | 0.80 ± 0.01 | 0.79 ± 0.03 | -0.012 | 159 (93.9 – 418.3) |
| | M30 | 18 | 9.33 ± 4.51 | 5.95 ± 0.96 | 5.88 ± 2.07 | 0.80 ± 0.02 | 0.79 ± 0.03 | -0.004 | - |
| 2015 | L30 | 9 | 6.80 ± 3.08 | 5.10 ± 0.69 | 5.78 ± 2.14 | 0.78 ± 0.04 | 0.80 ± 0.03 | 0.035 | - |
| | M30 | 24 | 9.33 ± 5.36 | 6.61 ± 1.03 | 5.93 ± 2.15 | 0.78 ± 0.02 | 0.79 ± 0.04 | 0.012 | - |
| 2017-2018 | L30 | 62 | 12.40 ± 7.09 | 7.02 ± 1.10 | 5.95 ± 1.93 | 0.80 ± 0.01 | 0.81 ± 0.03 | 0.012 | ∞ (393.4-∞) |
| | M30 | 83 | 13.00 ± 7.74 | 7.31 ± 1.31 | 5.94 ± 2.03 | 0.79 ± 0.01 | 0.80 ± 0.03 | 0.017 | 3668.9 (330.9 – ∞) |
| 2024 | L30 | 7 | 6.27 ± 2.37 | 4.64 ± 0.49 | 5.81 ± 2.07 | 0.77 ± 0.04 | 0.80 ± 0.03 | 0.037 | - |
| | M30 | 42 | 11.53 ± 6.96 | 6.99 ± 1.24 | 5.95 ± 2.11 | 0.80 ± 0.02 | 0.80 ± 0.03 | 0.000 | ∞ (540.6 – ∞) |
| 2012 | IR | 48 | 5.80 ± 2.37 | 3.85 ± 0.35 | 4.34 ± 1.22 | 0.75 ± 0.02 | 0.70 ± 0.04 | -0.074 | 8.2 (6.4 – 10.2) |
| 2016 | IR | 27 | 7.53 ± 3.48 | 4.39 ± 0.53 | 4.99 ± 1.46 | 0.76 ± 0.02 | 0.74 ± 0.03 | -0.020 | - |
| 2015 | NAR | 20 | 6.60 ± 2.59 | 4.15 ± 0.51 | 4.81 ± 1.38 | 0.73 ± 0.03 | 0.73 ± 0.03 | -0.001 | - |
| Total | | 408 | 15.33 ± 9.35 | 5.69 ± 0.28 | 5.84 ± 1.91 | 0.78 ± 0.01 | 0.80 ± 0.03 | | |

B.

| | Fish Group | | 1995 | | 2015 | | 2017-2018 | | 2024 | | 2012 | 2016 | 2015 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | L30 | M30 | L30 | M30 | L30 | M30 | L30 | M30 | IR | IR | NWAR |
| Wild | 1994-1997 | L30 | | 0.010 | 0.013 | 0.009 | 0.006 | 0.003 | 0.019 | 0.006 | 0.033 | 0.023 | 0.025 |
| | | M30 | ns | | 0.016 | 0.014 | 0.012 | 0.008 | 0.024 | 0.012 | 0.045 | 0.033 | 0.035 |
| | 2015 | L30 | ns | ns | | 0.015 | 0.014 | 0.013 | 0.026 | 0.014 | 0.045 | 0.033 | 0.031 |
| | | M30 | ns | ns | ns | | 0.007 | 0.007 | 0.022 | 0.011 | 0.045 | 0.031 | 0.032 |
| | 2017-2018 | L30 | ns | ns | ns | ns | | 0.004 | 0.019 | 0.006 | 0.040 | 0.028 | 0.031 |
| | | M30 | ns | ns | ns | ns | ns | | 0.020 | 0.005 | 0.036 | 0.027 | 0.029 |
| | 2024 | L30 | ns | ns | ns | ns | ns | ns | | 0.023 | 0.051 | 0.044 | 0.043 |
| | | M30 | ns | ns | ns | ns | ns | ns | ns | | 0.034 | 0.026 | 0.029 |
| Reared | 2012 | IR | * | * | * | * | * | * | * | * | | 0.016 | 0.020 |
| | 2016 | IR | * | * | * | * | * | * | * | * | * | | 0.013 |
| | 2015 | NWAR | * | * | * | * | * | * | * | * | * | ns | |

*: $p < 0.05$
ns: statistically not significant

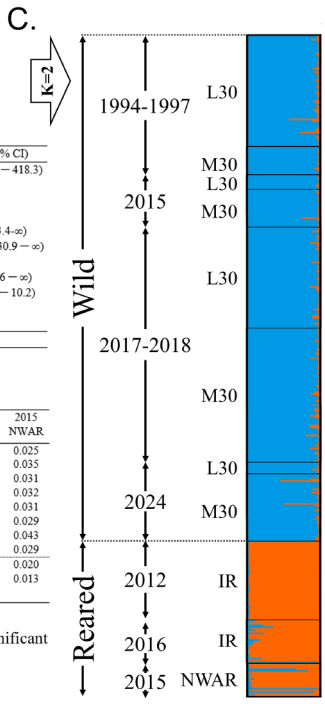


Figure 1. (A) Genetic diversity parameters for wild and farmed Gilthead seabream groups. n: number of individuals; *A*: mean number of alleles (±SD); *Ae*: effective number of alleles (±SE); *Ar*: allelic richness (±SD); *Ho* and *He* (±SD): observed and expected heterozygosity; $F_{IS}$: inbreeding coefficient; *Ne*: effective population size (CI: confidence interval). (B) Pairwise $F_{ST}$ values between wild and farmed groups. (C) Bayesian clustering analysis of genotypes based on 15 microsatellite loci with K = 2 (see text for details). L30: wild fish with degree of scale regeneration (SRD, % regenerated scales) ≤30%; M30: wild fish with SRD >30%; NWAR: farmed fish from the Northwestern Aegean Sea; IR: farmed fish from the Ionian Sea. Sample sizes smaller than 30 individuals were not used for the estimation of *Ne*.

Bayesian clustering analysis (STRUCTURE, K = 2) identified two distinct genetic clusters, clearly separating wild and farmed individuals, with no significant assignment of wild samples to the farmed cluster (Figure 1C). Overall, these results suggest that current escape events exert minimal detectable genetic impact on wild Gilthead seabream populations in the Ionian Sea.

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# PREVENTION OF VER IN SOLE USING PTS AND PTSO-SUPPLEMENTED DIETS

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## Introduction

Viral encephalopathy and retinopathy (VER), caused by nervous necrosis virus (NNV), is one of the main viral diseases affecting Senegalese sole (*Solea senegalensis*) in Southern Europe aquaculture. This virus (genus *Betanodavirus*, family *Nodaviridae*) is classified into four species: SJNNV, RGNNV, TPNNV and BFNNV (Sahul Hameed *et al*., 2019). In addition, highly virulent reassortants (RGNNV/SJNNV) have been identified in VER outbreaks in sole (Olveira *et al.,* 2009). Recently, NNV reassortant strain SsNNV19 was isolated from farmed Senegalese sole during an NNV outbreak in Southwestern Spain. Various prophylactic methods are being developed to reduce NNV infections in sole, including vaccines, supplemented functional feeds, and a combination of both treatments, among others. The *Allium* spp extracts propyl propane thiosulfinate (PTS) and propyl propane thiosulfonate (PTSO) are key compounds for the control of pathogenic microorganisms in livestock (Liu *et al.,* 2013), and their potential use in the aquaculture industry have been tested (Cabello-Gómez *et al.,* 2022). In this study, different sole feed supplemented with PTS or PTSO were developed, and its VER protective capacity was evaluated by measuring post-infection mortality and the study of host oxidative stress and inflammatory response after the feeding regime.

## Materials and methods

Sole (0.5g) were divided into three groups and fed with different WinFlat (SPAROS) based feed formulations: Control (WinFlat), PTS (WinFlat + PTS), and PTSO (WinFlat + PTSO) at 6% of biomass for 30 days. At the end of the feeding period, six fish from each experimental group were sacrificed, and gene expression related to oxidative stress in the liver and inflammatory response and apoptosis in the intestine were analysed by qPCR. After feeding, the fish were experimentally infected with the SsNNV19 strain by intramuscular injection at $10^5$ $TCID_{50}$/g of fish. Mortality was recorded for 30 days.

## Results

In Senegalese sole specimens fed with PTSO-supplemented diet, downregulation of the *hsp90* (-3.32) and *nos* (-2.89) genes was observed, as well as upregulation of the *sod* (1.17) gene in the liver. In the case of the specimens fed with PTS-supplemented diet, only the differential expression of *sod* (1.035) gene was observed, being upregulated (Figure 1).

In the intestine, no significant differences were observed in the expression of genes related to the inflammatory process. However, in specimens fed with both supplemented diets (PTSO and PTS), upregulation of *casp9* (0.88 and 0.83) gene, related to the cell apoptosis process, was observed (Figure 2).

During the first days of the challenge, mortality registered in fish fed with the supplemented diets was lower than in the control group, but from 9 dpi onward, mortality increased similarly in all groups, ending with 100% mortality at 19 days post-challenge.

## Conclusions

Dietary supplementation with PTS and, in particular, PTSO modulates the expression of genes related to oxidative stress in the liver, which could lead to an improvement in the health status of Senegalese sole specimens.

In intestinal samples, no induction of genes related to the inflammatory response was observed after 30 days of feeding with the supplemented diets. Nevertheless, the induction of the gene encoding caspase-9, associated with apoptosis, in both diets could indicate a certain degree of intestinal toxicity of these compounds.

Although these compounds do not confer direct protection, they may be used in conjunction with other strategies, such as vaccination, since the mitigation of oxidative stress, together with the reduction of early mortality, can potentiate the efficacy of vaccines.

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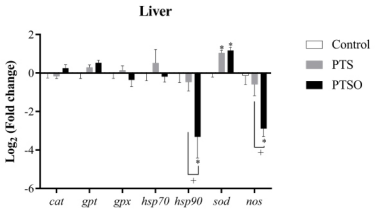


Fig1. Differentially expressed genes related to oxidative stress in liver samples of Senegalese sole fed with PTSO and PTS-supplemented diets. Values are mean ± SD (n = 6). * denote significant differences between control and treatment group ($p < 0.05$). $^{+}$ denote significant differences between treatments ($p < 0.05$).

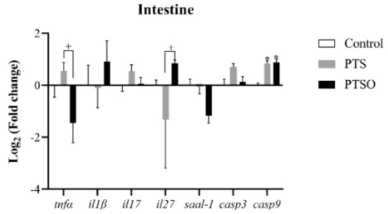


Fig2. Inflammatory response and apoptosis related gene transcription in the intestine of Senegalese sole fed with PTS and PTSO. Values are mean ± SD (n = 6). * denote significant differences between control and treatment group ($p < 0.05$). $^{+}$ denote significant differences between treatments ($p < 0.05$).

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# ENHANCING IMMUNITY, HEALTH AND PERFORMANCE IN ATLANTIC SALMON (*Salmo salar*) FRY AND REDUCING THERMAL STRESS IN RAINBOW TROUT (*Oncorhynchus mykiss*) THROUGH DIETARY SUPPLEMENTATION WITH ALGAL 1,3-β-GLUCAN: INSIGHTS INTO SUSTAINABLE AQUACULTURE PRACTICES

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**Introduction**

The intensification of aquaculture practices has heightened the need for sustainable strategies to improve fish health and reduce dependency on antibiotics. Atlantic salmon (*Salmo salar*), a cornerstone species in global aquaculture, is particularly susceptible to stress and disease under intensive farming conditions. Recent research has highlighted the immunomodulatory potential of natural compounds, such as 1,3-β-glucans derived from microalgae, which may enhance host defense mechanisms and overall physiological resilience. As a functional feed additive, algal 1,3-β-glucan (ABG) represents a promising approach to improve immune responses, growth performance, and survival rates in salmon aquaculture, aligning with the industry's shift toward more sustainable and health-oriented production systems.

This study evaluated the effects of dietary algae-derived 1,3-β-glucan supplementation on growth performance, immune response, and disease resistance in Atlantic salmon (*Salmo salar*). A total of 360 fish were assigned to control and treatment groups receiving 250–1000 g/ton of algal 1,3-β-glucan over six months. Key parameters assessed included condition factor, immune activity, and resistance to *Aeromonas salmonicida* (Furunculosis). In addition, effect of algal 1,3-β-glucan on chronic thermal stress in rainbow trout (*Onchorhynchus mykiss*) have been assessed.

**Materials and methods**

This six-month study evaluated the effects of dietary algal 1,3-β-glucan supplementation on growth, immunity, and disease resistance in Atlantic salmon fry (6–8 inches). All experiments were conducted at 10–12°C in a controlled environmental chamber.

Phase I (Days 0–14): Fish (n=360) were acclimated in twelve 30-gallon tanks (30 fish/tank), with standard feed provided twice daily. Length and weight measurements were recorded on days 0 and 14 to assess baseline condition.

Phase II (Days 15–45): Fish were divided into four treatment groups: Control (no β-glucan), and three algal 1,3-β-glucan doses - 250, 500, and 1000 mg/kg feed. Condition factors, immune parameters, and hematological indices were assessed on days 30 and 45.
Phase III (Days 46–75): Fish were challenged with *Aeromonas salmonicida* ($1 \times 10^5$ CFU/fish) via intraperitoneal injection. Positive controls received the pathogen, while negative controls received PBS. Daily mortality was recorded for 30 days. Additional immune and hematological assessments were conducted on days 60 and 75.

Health and Immune Assessment: Growth and condition were monitored via weight gain, length:weight ratio, and condition factor. Hematological parameters including red and white blood cell counts, hematocrit, and hemoglobin were measured using hematological assays.

Thermal stress study: Efficacy of algal 1,3-β-glucan on reducing heat stress was assessed by measuring the scale cortisol level. Treatment groups involved negative control, cortisol control (CORT 500), 250 ppm water soluble (WS) algal 1,3-β-glucan.

**Results**

Survival rate was linearly correlated with increasing doses of algal 1,3-β-glucan. The highest survivability was observed in the algal 1,3-β-glucan at 1000 g/MT (90%), compared to 46% in the control group.

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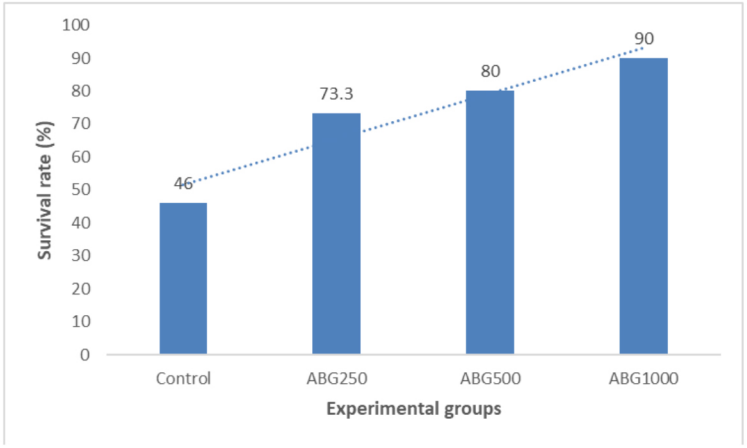


**Fig. 1: Survival rates among control and algal 1,3-β-glucan (ABG) treated groups**

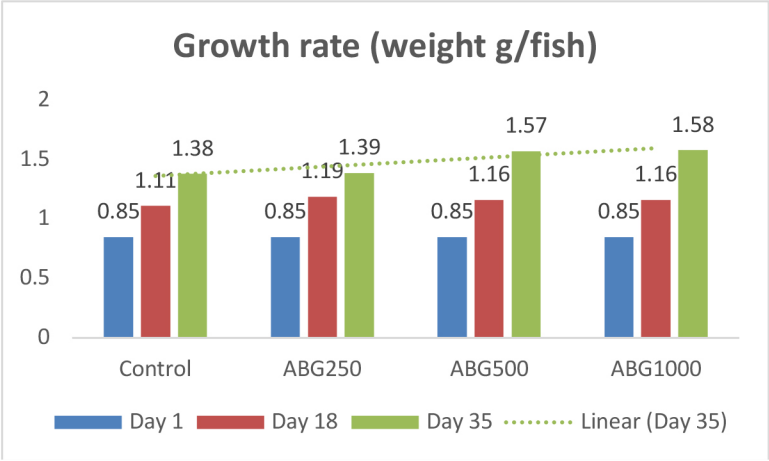


**Fig. 2: Growth rate of fish among control and algal 1,3-β-glucan (ABG) treated groups**

A significant improvement in the growth was observed in the algal 1,3-β-glucan treated groups (500g/MT and 1000g/MT). There were no statistically significant differences observed in hematocrit, or red blood cell counts among treatment groups. These findings support the potential of algal 1,3-β-glucan as a functional feed additive in sustainable salmon aquaculture.

The scale cortisol level at the start was on average 0.056±0.040 µg/kg. After 3 weeks, the scale cortisol in CORT500 group had increased. The variation in scale cortisol between individual fish is smaller in the groups given WS 1,3-β-glucan than in the control or CORT 500 group. This is an indication that the algal beta-glucan helps tuning the HPI axis activation during the first three weeks.

**Conclusion**

The study demonstrates that algal-derived 1,3-β-glucan is an effective immunostimulant and growth promoter in Atlantic salmon and thermal stress management tool in trouts. Its incorporation into commercial diets has the potential to improve fish health and performance while reducing reliance on chemotherapeutics. These findings support the broader adoption of microalgal bioactive in sustainable aquafeed formulations.

# NON-INVASIVE MONITORING OF FISH MORPHOMETRIC TRAITS USING YOLO-BASED COMPUTER VISION IN AQUACULTURE

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## Introduction

The aquaculture industry has grown substantially over the past 25 years, with production increasing by 200% and global fish trade by 300% since 1995 (FAO, 2021). As the fastest-growing food production sector, aquaculture requires efficient and sustainable practices that support both productivity and animal welfare. Key anatomical features such as the pectoral fins, tail, and skin are critical for fish locomotion, posture, and health, yet are highly vulnerable to damage caused by handling, high stocking densities, and underlying health issues. Such impairments can adversely impact welfare, feeding efficiency, and growth. Despite their importance, the condition of these features remains largely under-monitored due to the reliance on manual assessment methods, which are time-consuming, labor-intensive, and potentially harmful to fish. This work aims to develop and validate non-invasive computer vision models for automatically monitoring fish morphometric traits—including fins, tails, and skin—and for classifying their condition as 'good' or 'bad.' The objective is to provide an efficient and reliable tool to improve health monitoring and welfare management in aquaculture systems.

## Materials and methods

Two YOLO architectures—YOLOv8 and YOLOv12—were trained to detect three morphometric characteristics (tail, pectoral fin, and red skin spots) in two fish species: European seabass and gilthead seabream. Data were collected using a stereo vision system (1080p resolution) with a patented software (Voskakis et al., 2021) in circular polyester cages (40 m diameter, 9 m depth) at HCMR's pilot farm in Souda Bay, Crete (certified: GR94FISH0001). Images were annotated into "good" and "bad" classes for tails and fins, and a single class for red skin spots. Training datasets included 2,383 fin images (gilthead seabream), 1,414 fin images (European seabass), 5,041 tail images (gilthead seabream), 4,276 tail images (European seabass), and 584 skin images with red spots for both species. Models were trained using Python on an NVIDIA RTX 4080 GPU (batch size: 12, image size: 640×640), with early stopping (patience = 30) to prevent overfitting. Extensive data augmentation strategies for underwater object detection were tested. A total of over 125 training runs were conducted as datasets were incrementally expanded.

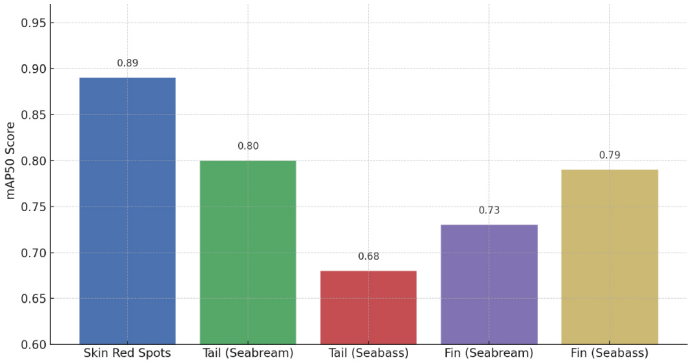


Fig. 1. Yolov12 detection performance by morphometric feature and species.

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**Results**

YOLOv8 and YOLOv12 both performed well in detecting morphometric features across species, with YOLOv12 showing superior computational efficiency and less overfitting. Transitioning from YOLOv8 Large to YOLOv12 Small model led to a **78.7% reduction in parameters** (43.7M → 9.3M) (Jocher et al., 2021; Tian et al., 2025) without loss in detection accuracy. Despite its smaller size, YOLOv12 Small matched or outperformed YOLOv8 Large. In terms of detection performance, detection of red skin spots achieved the highest accuracy, with a **mean Average Precision at IoU 0.5 (mAP50)** of **0.89**. Tail condition classification in **gilthead seabream** reached **mAP50 = 0.80**, while in **European seabass**, it achieved **0.68** (Figure 1). For **pectoral fin classification**, the seabream model reached **mAP50 = 0.73**, whereas the **seabass model performed better**, achieving **mAP50 = 0.79**.

**Conclusion**

Overall, detection accuracy varied across species, morphometric traits but also from differences in image quality due variable environmental conditions. Red skin spots were the most accurately detected feature, while tail condition in European seabass showed the greatest need for further improvement. Both YOLO models performed reliably; YOLOv12 demonstrated advantages in computational efficiency. Future steps include to test the system on large scale aquaculture farms by estimating the percentage of damaged morphometric features.

**Acknowledgement**

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# DETERMINATION OF OPERATIONAL WELFARE INDICATORS AS A TOOL FOR A SUSTAINABLE AQUACULTURE OF THE COMMON OCTOPUS, *Octopus vulgaris*

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## Introduction

Animal welfare is a growing public concern, especially for the emerging field of octopus aquaculture. The common octopus, *Octopus vulgaris,* is a commercial valuable species, and an excellent candidate for aquaculture diversification (1). Overfishing of natural stocks and high market demand make sustainable octopus aquaculture essential to reduce fishing pressure and protect natural populations. To achieve this, optimizing and standardizing practices to control and safeguard animal welfare is crucial. Welfare assessment should prioritize aspects such as diet, appropriate housing conditions, environmental enrichment, behavior, and health. In this context, the early detection of pathogens, particularly the protozoan parasite *Aggregata octopiana* and bacteria of the *Vibrio* genus, is essential to prevent disease outbreaks and reduce animal suffering (2-3). Moreover, the identification of protein- or microbiota- based welfare markers from non-invasive samples, such as skin mucus, could provide powerful tools for routine health monitoring, offering insight into the physiological status of the animals without compromising their welfare. This study identifies specific octopus welfare markers, defined as biological Operational Welfare Indicators (OWIs), to be integrated into monitoring plans for good aquaculture practices.

## Material and methods

Quantitative PCR (qPCR) assays were developed for the absolute quantification of the coccidian *A. octopiana* in fecal samples, using standard curves based on a plasmid-cloned target 18S rDNA sequence with known copy numbers. qPCR results were correlated with microscopic examination of fecal smears, applying an optimized algorithm in Python 3.11.8 that selected threshold pairs from qPCR Ct values and microscopy count, allowing the establishment of different infection ranges of infection. A similar qPCR approach was used to quantify *Vibrio* spp. from tank seawater and skin mucus samples, employing genus-specific primers, and results were validated through colony counts on selective TCBS agar. A shotgun proteomics approach combined with liquid chromatography coupled with tandem mass spectrometry (LC-ESI-MS/MS) using an Orbitrap-Elite instrument was used to create a reference dataset from octopus skin mucus including wild and aquarium maintained octopus, as well as adult and senescent ones. The final proteome compilation was investigated by integrated *in silico* studies, including Gene Ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, network studies, and prediction and characterization analysis of potential bioactive peptides. Additionally, tank seawater and skin samples from individuals showing external lesions were subjected to shotgun metagenomics sequencing (RNA-seq Illumina) to analyze microbial community structure and potential pathogens associated with compromised skin integrity. All samples were collected from octopuses under aquaculture conditions.

## Results

Absolute quantifications of *A.octopiana* and *Vibrio* spp by qPCR enabled rapid, reliable, and low-impact monitoring of infection in aquarium-maintained octopuses, supporting early diagnosis and improved animal welfare. The quantification of *A. octopiana* 18S rDNA by qPCR using plasmid-based standard curves achieved a high efficiency (97%, $R^2 = 0.995$) and a detection limit of $10^2$ copies/µL. The assay showed strong repeatability (CV = 0.01) and high specificity. The comparison between data obtained by qPCR and those obtained with microscopy counts using an optimized algorithm let to define three infection levels (low, medium, high), yielding a good correlation (r = 0.723) between both methods. This allowed reliable, quantitative categorization of parasite load in non-invasively collected samples. In addition, absolute quantification of *Vibrio* spp. was successfully implemented using genus-conserved primers in a qPCR assay. The correlation between Ct values obtained by qPCR and colony counts on selective TCBS agar validated the method and enabled the identification of potentially pathogenic

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*Vibrio* colonies in culture conditions. Proteomic analysis revealed a different pattern between conditions. Although similar profiles were observed, differences in relative abundances led to the identification of potential biomarkers, including caspase-3-like, protocadherin 4, deleted in malignant brain tumors, thioredoxin, papilin, annexin, cofilin and mucin-4 proteins. Some of these proteins also revealed potential as bioactive peptides, with several displaying antibacterial and antiviral activities in skin mucus, as identified using the CAMP (Collection of Anti-Microbial Peptides) database. The metagenomics analysis identified different bacterial species corresponding mainly to the genus Vibrio as causative of skin ulcerations.

**Discussion**

Ensuring animal welfare in aquaculture systems is essential, and the identification of reliable OWIs is key to achieving this goal. Although octopus farming is not yet established at an industrial scale, preserving welfare in aquarium-maintained individuals is crucial, both for ethical husbandry and as a base for future large-scale aquaculture practices. This study highlights the effectiveness of non-invasive molecular tools for monitoring the health and welfare of *O. vulgaris* under aquarium conditions. The validated qPCR assays for *A. octopiana* and *Vibrio* spp. enabled sensitive, repeatable, and early detection of infections, supporting their use as practical OWIs. The strong correlation between molecular and traditional diagnostic methods further reinforces their reliability.

Similar approaches using absolute qPCR have proven effective in other species for pathogen detection and infection level assessment (4-5), further supporting the value of these tools for developing standardized, cross-species welfare monitoring protocols in aquaculture. Additionally, while metagenomics analysis provided insights into microbial communities associated with skin lesions, proteomic profiling of skin mucus allowed exploration of how aquarium maintenance conditions and senescence alter the mucus proteome. Also, it revealed a wide array of bioactive peptides with potential antimicrobial and immunological roles, which, similar to findings in fish and other molluscs, can serve as reliable indicators of physiological status and stress (6), supporting their potential use as OWIs in octopus. These findings highlight skin mucus as a promising, non-invasive source of biomarkers for health and welfare monitoring in octopus aquaculture.

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# EFFECT OF SEASON AT SEAWATER TRANSFER ON ATLANTIC SALMON GUT MICROBIOME AND ITS INTERACTION WITH PERFORMANCE IN FLOW-THROUGH SYSTEMS

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## Introduction

Seawater transfer (SWT) represents a critical phase in Atlantic salmon aquaculture, often accompanied by high stress and susceptibility to infections, which are influenced by various environmental factors, including seasonality. The gut microbiome is essential for fish health, influencing digestion, immunity, and barrier functions. Nevertheless, the impact of the season at seawater transfer on microbiome composition and subsequent fish performance in flow-through systems remains understudied. This research investigates seasonal effects (spring vs autumn) at the time of SWT on gut microbiome dynamics and their implications for salmon performance.

## Materials and methods

Atlantic salmon smolts were transferred from freshwater to seawater during two different seasons: spring and autumn. Sampling occurred at multiple timepoints: just before SWT (Code 3 for spring; Code 7 for autumn), one week after SWT (Code 4 spring; Code 8 autumn), and six months post-transfer (Code 5 spring; Code 9 autumn). Feces samples were collected from the posterior intestine, and genomic DNA was extracted. Microbiome composition was assessed via 16S rRNA gene sequencing (V3-V4 regions), using Illumina technology. Bioinformatic analyses included quality filtering, ASV assignment (DADA2), taxonomic classification (SILVA138.1 database), and diversity analyses (Shannon and Bray-Curtis indices).

## Results

Gut microbiota showed significant seasonal differences at seawater transfer, with higher microbial diversity observed during autumn compared to spring (Shannon index, $p<0.05$). In spring-transferred smolts, low diversity correlated with increased abundance of the genus *Brevinema*. Bray-Curtis analysis indicated distinct microbiota compositions between seasons (MANOVA, $p.adj < 0.001$), suggesting a more specialized microbiota over time in autumn cohorts. At later stages, *Malacoplasma* emerged as the dominant genus in both seasonal cohorts. Furthermore, specific taxa such as *Photobacterium* and *Carnobacterium* varied significantly between seasonal transfers, implicating potential functional implications for fish health.

## Discussion and conclusion

Our findings indicate clear seasonal effects on gut microbiome composition at seawater transfer, with autumn transfers supporting higher microbiota diversity and specialization. Such microbiota differences may underlie differential performance outcomes and disease resistance. Seasonal variation in microbial communities underscores the importance of considering environmental timing in aquaculture practices. Future studies should investigate functional implications and potential microbial biomarkers such as *Brevinema* for optimizing salmon production in flow-through systems.

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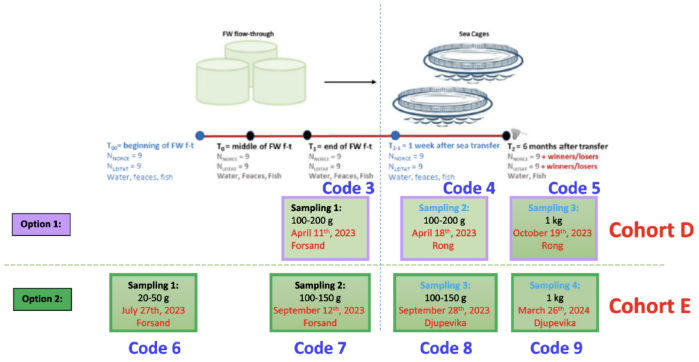


Fig. 1. Sampling of the salmon cohort across life stages and production environments.

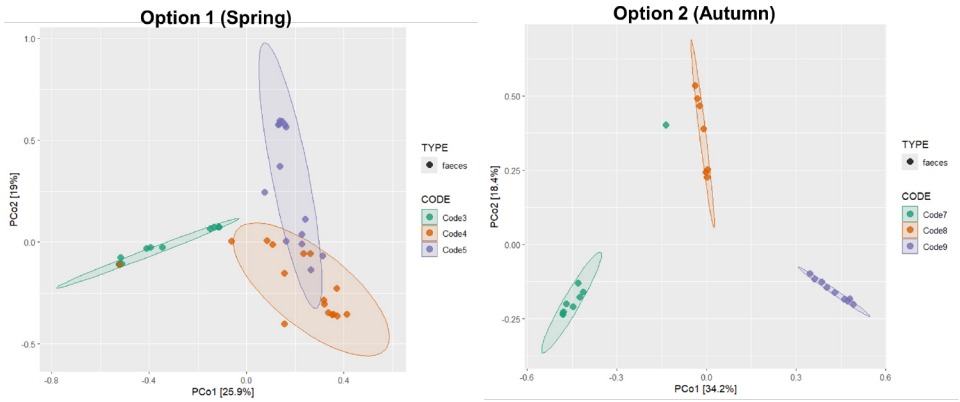


Fig. 2. Beta diversity across sampling points based on Bray Curtis dissimilarity. Samples are switched between Option 1 (Spring) and Option 2 (Autumn)

**Acknowledgments**

This project has been funded by BlueRemediomics-Grant No.101082304, EU Horizon Europe Programme 2022. Fish were sampled from Lerøy´s land and sea facilities in Norway.

# EFFECT OF RAS ON ATLANTIC SALMON GUT MICROBIOME AND ITS INTERACTION WITH PERFORMANCE AFTER SEAWATER TRANSFER

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## Introduction

Recirculating Aquaculture Systems (RAS) are increasingly common land-based production systems, however, biological requirements of the fish are not always considered, contributing to the high mortalities observed, notably following the seawater transfer (SWT). SWT is a challenging production phase associated with stress, impaired welfare, and elevated susceptibility to opportunistic infections (Sommerset et al., 2023). The gut microbiome plays an array of critical roles in fish biology, not only during digestion but also in the immune and barrier functions with large implications for the fish's health. Importantly, little work has been conducted to investigate how farming procedures can alter the fish' microbiome and the health of the holobiont. This study aims to characterize the effects of different RAS protocols (size at SWT) on gut microbiome, in relation to fish health and welfare.

## Materials and methods

A fish cohort from a commercial production facility was monitored from the parr stage in freshwater (FW) RAS through transfer to brackish water (BW) RAS. Two transfer strategies were applied: release of small (150 g) or large (500 g) smolts to sea cages (SC). Sampling points are depicted in Fig. 1 and were as follows: A) FW RAS: parr at 50 g **(Code 0)** and 100 g just before transfer to BW **(Code 1)**; B) BW RAS: one week after transfer **(Code 2)** and just before seawater transfer (SWT) at either 100 g **(Code 3)** or 500 g **(Code 6)**; and C) SC: at one week **(Codes 4 & 7)** and six months after SWT **(Codes 5 & 8)**. Fish weight and somatic indexes were recorded. Three sample sets were collected: plasma for general welfare assessments and water and feces for microbiome analysis. Feces were collected from the posterior intestine. Total genomic DNA was extracted for metataxonomic, DNA amplicon libraries were generated targeting the V3-V4 regions (341F/R805) of the 16S rRNA gene, and the sequencing was performed on the Illumina platform. Analysis of the sequences was performed following standards pipelines for metataxonomic analysis. Clean sequences chimeric detection and deletion, followed by Amplicon Sequence Variant (ASV) assignment was completed using the DADA2 plugin. Taxonomy was assigned with a 99% similarity level using the q2-feature-classifier plugin with the SILVA138 database. Alpha (Shannon) and beta (Bray Curtis) diversity indices were calculated using the vegan R package.

## Results

Alpha-diversity was low in the salmon gut microbiota at the early parr stage (Code 0) (Fig. 2A) and closely resembled the RAS water, being largely dominated by *Aurantimicrobium* (data not shown). From the smolt stage onwards (Code 1), the microbiota became more diverse and increasingly distinct from the surrounding water, despite the BW being less diverse than the FW. Beta-diversity revealed significant differences among Codes 0, 1, and 2, with the most pronounced difference observed between Codes 1 and 2 (Fig. 2C).

In the remaining samples, alpha-diversity was significantly different between salmon gut microbiota one week after SWT in fish transferred at 150 g and 500 g (Codes 4 and 7; Fig. 2B). According to beta-diversity analysis, significant differences existed between small and large smolts during the BW phase (Codes 3 and 6; Fig. 2D). SWT of smaller fish was associated with higher gut microbiota diversity compared to fish transferred at bigger size. Gut microbiome differences were most pronounced one week after sea transfer (Codes 4 and 7). By the end of the sea-cage phase (Codes 5 and 8), differences were less marked but remained significant (Fig. 2D) with *Mycoplasma* emerging as the dominant genus (data not shown). Correlation analysis

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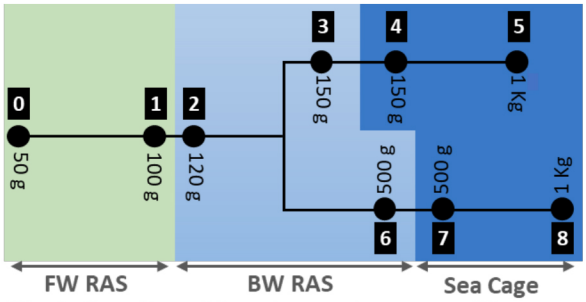


Fig. 1. Sampling of the salmon cohort across life stages and production environments. Sampling codes are marked with black squares

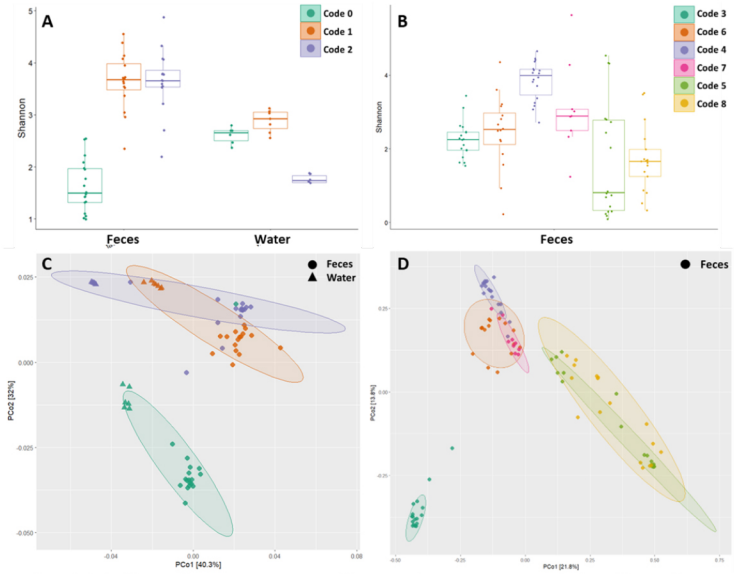


Fig. 2. Microbial diversity across sampling points. (A, B) Alpha-diversity measured by the Shannon index. (C, D) Beta-diversity based on Bray-Curtis dissimilarity.

between gut microbiota and fish weight revealed significant associations, particularly in the cohort transferred at smaller size (data not shown). Most correlations were positive (e.g., *Prochlorococcus*, *Aeriscardovia*, and *Mycoplasma*), while a few were negative (e.g., *Francisella*). Correlation with other biological aspects is in progress and will be updated at a later stage before the presentation.

**Discussion and conclusion**

Our findings show that the salmon gut microbiota gradually diverges from the surrounding environment and becomes specialized over time. Although gut communities converged by the end of the sea production cycle, differences in body weight at SWT appear to shape microbial composition of the early SW phase, likely contributing to the heightened vulnerability and mortality often observed at this stage. Notably, the association between microbiota profiles and fish size at SWT suggests a potential link to performance. Further research is needed to clarify how microbial shifts over time relate to other biological factors. Overall, our results highlight life stage–dependent changes in gut microbiome and suggest functional relevance, an area still underexplored in fish microbiome research.

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**Acknowledgments**

This project has been funded by BlueRemediomics-Grant No.101082304, Horizon Europe Programme 2022. Fish were sampled from Lerøy´s land and sea facilities in Norway.

# BLACK SPOT INHIBITION AND FRESHNESS PRESERVATION USING A SULFITE-FREE ADDITIVE IN HARVESTED SHRIMP

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This study evaluates the efficacy of PrawnFresh™ , a sulfite-free antimelanosic agent developed by Xyrex Ltd, in preserving the appearance and freshness of Penaeus vannamei and Penaeus monodon. Comparative trials were conducted to assess melanosis inhibition, microbiological quality, and overall shelf life in shrimp treated with PrawnFresh™ versus untreated controls. Results demonstrated that PrawnFresh™ effectively inhibited melanosis, maintaining 0% discoloration after 90 days of frozen storage at -18°C, whereas untreated samples exhibited melanosis levels exceeding 40%. Additionally, microbiological assessments indicated that PrawnFresh™ extended shrimp shelf life by 6–8 days beyond conventional methods, without compromising sensory attributes. These findings suggest that PrawnFresh™ is a viable and sustainable alternative to sulfite-based treatments, aligning with regulatory requirements and industry demands for improved seafood preservation.

**Introduction**

Melanosis is a major post-harvest challenge in shrimp and other crustaceans, leading to product degradation and reduced consumer appeal. This enzymatic process is primarily catalyzed by polyphenol oxidase (PPO), which, upon exposure to oxygen, oxidizes phenolic compounds to quinones that polymerize into dark pigments. While melanosis does not pose a food safety risk, its occurrence negatively impacts the seafood industry by decreasing product value and shelf life. Sulfite-based treatments, particularly sodium metabisulfite (SMS), have traditionally been employed to inhibit PPO activity; however, their use raises several concerns. SMS can produce toxic fumes, cause allergic reactions in sensitive individuals, corrode processing equipment, and contribute to aquatic contamination through processing effluents. Regulatory shifts and consumer demand for cleaner-label alternatives have driven the exploration of novel sulfite-free melanosis inhibitors. PrawnFresh™, a proprietary additive formulated by Xyrex Ltd, presents an innovative solution that mitigates these drawbacks while ensuring high-quality shrimp preservation.

**Objective**

- To evaluate the efficacy of PrawnFresh™ in inhibiting melanosis in harvested shrimp.
- To compare the effects of PrawnFresh™ with traditional sulfite treatments and untreated controls.
- To assess the microbiological impact of PrawnFresh™ on shrimp quality and shelf life.

**Methodology**

Post-harvest trials were conducted using Penaeus vannamei and Penaeus monodon. Shrimp were subjected to a chill-kill process immediately after harvest, immersed in an ice-water slurry containing PrawnFresh™, and maintained under strict cold chain conditions. Samples were divided into treatment and control groups, with the control group receiving no antimelanosic agent.

Melanosis progression and microbiological quality were evaluated at 48 hours, 15 days, 30 days, 60 days, and 90 days of frozen storage at -18°C. Additionally, a subset of samples was stored at 4°C for up to 12 days to assess melanosis under refrigerated conditions. Each treatment group was analyzed in triplicate to ensure statistical reliability. Microbiological assessments focused on total viable count (TVC), psychrotrophic bacteria, and specific spoilage organisms.

**Results**

- PrawnFresh™ -treated shrimp exhibited complete inhibition of melanosis (0%) after 90 days at -18°C, whereas untreated samples showed melanosis levels exceeding 40%.
- Under chilled storage at 4°C, melanosis in untreated samples reached 100% by day 4, while PrawnFresh™ -treated shrimp displayed minimal browning until day 12.
- Microbiological analysis indicated that PrawnFresh™ extended shrimp shelf life by an additional 6–8 days compared to untreated samples, maintaining acceptable TVC levels and sensory attributes.

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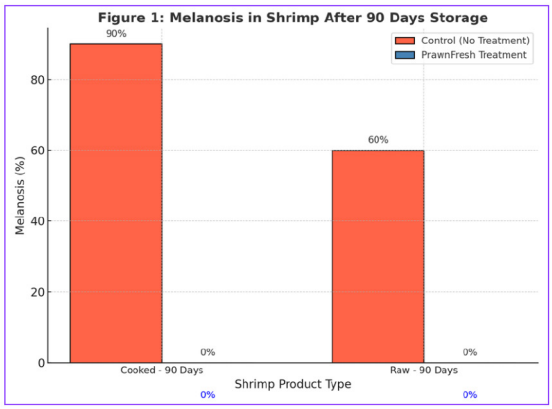


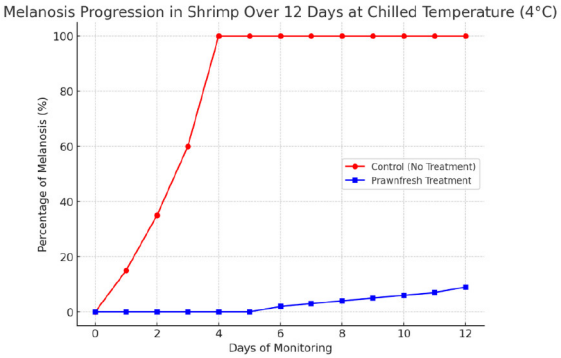


Figures:

1. **Melanosis inhibition in frozen shrimp:** Comparison of control and PrawnFresh™-treated samples over 90 days of storage at -18°C. ***Figure 1 illustrates*** the effectiveness of PrawnFresh in preventing melanosis in shrimp after 90 days of storage. The control group (A) shows high melanosis levels (90% in cooked shrimp and 60% in raw shrimp), whereas the treated group (B) exhibited no melanosis (0%) in both conditions
2. **Melanosis progression in chilled shrimp:** Monitoring of black spot development in treated vs. untreated samples at 4°C over 12 days. Melanosis progression monitored over 12 days at a chilled temperature of 4°C for two treatments: a control group (no treatment) and a Prawnfresh-treated group. The control group exhibited a rapid increase in melanosis, reaching 100% by day 4 and remaining stable until day 12. In contrast, the Prawnfresh treatment effectively delayed melanosis,

**Discussion**

The results demonstrate that PrawnFresh™ effectively inhibits melanosis in shrimp, offering a sulfite-free alternative that meets industry and regulatory standards. The absence of melanosis in treated samples after prolonged frozen storage underscores its potential as a superior preservation method compared to conventional SMS treatments. Furthermore, the observed extension of microbiological shelf life suggests that PrawnFresh™ functions as both an antimelanosic and a preservative, improving overall product stability.

From a commercial perspective, PrawnFresh™ addresses several industry challenges, including worker safety, environmental sustainability, and regulatory compliance. Its adoption could enhance operational efficiency by reducing corrosion-related equipment maintenance and minimizing chemical waste in aquaculture environments.

**Conclusion**

PrawnFresh™ emerges as a promising sulfite-free alternative for shrimp melanosis inhibition and quality preservation. By effectively preventing black spot formation and extending shelf life without adverse sensory or microbiological effects, it provides a sustainable and regulatory-compliant solution for seafood processors. These findings support the broader adoption of sulfite-free treatments in response to evolving industry demands and environmental concerns.

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***This figure*** illustrates the effectiveness of PrawnFresh in preventing melanosis in shrimp after 90 days of storage.

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# BIOCHEMICAL RESPONSE OF JUVENILE *Dicentrarchus labrax* TO NOISE GENERATED BY MARITIME TRAFFIC

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**Introduction**

Aquaculture plays a key role in global food security and is expanding rapidly to meet the demands of a growing population (Verdegem et al., 2023). However, its sustainability is challenged by environmental impacts and stressors affecting fish welfare. Among these, underwater noise—often overlooked—can disrupt fish physiology, behavior, and immune function (Wysocki et al., 2007; Kunc et al., 2016; Filiciotto et al., 2017). Despite increasing awareness, data on its effects in aquaculture settings remain scarce (Duan et al., 2025).

In the Mediterranean, European seabass (*Dicentrarchus labrax*) is a key aquaculture species due to its adaptability and commercial value (Varsamos et al., 2001). This study examines the short- and long-term physiological impacts of acoustic exposure on juvenile *D. labrax*, aiming to support more welfare-focused and sustainable farming practices.

**Material and Methods**

The experiment was conducted at the facilities os the Universitat Politécnica de Valencia in the port of Gandia. Using a total of 144 juvenile of Dicentrarchus labrax, placed in tanks (147 cm x 118 cm x 89 cm). The animals were subjected to acoustic stress for 30 minutes, 3 hours, 24 hours, and 7 days. For each time point, three replicates were performed.

An electroacoustic setup was developed to generate underwater noise within the tanks, comprising a digital acquisition card (DAQ), a Sony FA-TE370 amplifier, and a custom UA-UPV speaker positioned inside the tank without contact with the walls. The speaker was calibrated using a reference hydrophone. Acoustic stimuli consisted of randomized recordings from four fishing vessels (10–15 m distance), lasting 90–150 s, with 10–30 s silent intervals. Emissions were primarily in the low-frequency range (50–2000 Hz).

At each sampling time, fish were stored at −20 °C. Whole-body extracts in PBS were analyzed for cortisol, HSP70, SOD, GPx, and ACTH via ELISA. Glucose was measured with a glucometer, while alkaline phosphatase and esterase activities were determined using modified colorimetric protocols. Enzymatic reactions were run at 30 °C, monitored at 405 nm, and expressed as U/μg protein.

**Results**

Short-term acoustic exposure (30 minutes) led to significant increases in all measured stress and oxidative biomarkers. HSP70, SOD, GSH-PX, ACTH, cortisol, esterase, alkaline phosphatase, and glucose levels all rose markedly in response to the initial acoustic stimulus.

HSP70 and ACTH levels remained significantly elevated after 3 hours but returned to baseline after 24 hours and 7 days. SOD and GSH-PX also showed elevated values at 30 minutes and 3 hours, with only GSH-PX remaining significantly higher at 24 hours; both returned to control levels by day 7.

Cortisol levels peaked at 3 hours post-exposure—unlike the other parameters—and normalized by day 7. Glucose levels also peaked at 30 minutes, remained elevated at 3 hours and 7 days, but returned to control levels at 24 hours.

Esterase activity showed an early increase at 30 minutes, followed by a decline to baseline levels from 3 hours onwards. Alkaline phosphatase peaked at 30 minutes and gradually decreased at subsequent time points, but did not return to control levels even after 7 days.

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**Conclusion and Discussion**

Early life stages of fish are highly sensitive to environmental stressors, making the study of acoustic pollution particularly relevant. In Mediterranean aquaculture, where *Dicentrarchus labrax* is economically important, assessing physiological responses to sound can inform welfare-oriented practices. Whole-body analyses of juvenile seabass revealed short- to medium-term changes in key cellular markers, indicating a strong acute stress response to acoustic exposure, with varying recovery times depending on the biomarker evaluated and potential compensatory mechanisms that enhance coping capacity.

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# TOWARDS FULLY AUTOMATED VIDEO ANALYSIS IN ECOLOGICAL AND AQUACULTURE BEHAVIOURAL STUDIES

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**Introduction**
The SONORA project aims to evaluate the effects of underwater noise on commercially relevant fish species, focusing on growth, biochemical parameters, and behavior. Behavioral analysis requires processing large volumes of video recordings, which makes manual evaluation impractical and resource-intensive. This study explores the potential of artificial intelligence (AI) and computer vision to automate the detection and tracking of fish, enabling large-scale and precise behavioral analysis in aquaculture and ecological experiments.

**Material and Methods**
Multiple publicly available dorsal-view fish datasets were compiled, totaling approximately 35,000 images and 1 million annotations. A baseline fish detection model was trained using YOLOv8, achieving a mean Average Precision (mAP) of 0.94 for dorsal-view detection. An experimental setup was then implemented with 20 juvenile European seabass (*Dicentrarchus labrax*), in which 5 hours of video footage were recorded. The model was fine-tuned using only 20 annotated images from this setup.

**Results**
The fine-tuned YOLOv8 model achieved a detection accuracy of 0.98 mAP in our experimental setup. The high detection performance enabled robust fish tracking with the BoT-SORT algorithm, allowing for the automatic computation of first- and second-order behavioral metrics: detection distribution maps, distance to the sound source, shoal cohesion, 2D swimming speed, and swimming direction. This metrics allows us to observe differences between fish exposed to different acoustic stimuli and control groups.

The system is being applied in a 3-month behavioral experiment with 6 tanks equipped with a multi-camera recording system, capturing 8-10 hours of video daily.

**Conclusion and Discussion**
This study approximates the use of AI-based computer vision systems to automate the detection, tracking, and behavioral analysis of fish in aquaculture tanks. The main limitations are related to detecting very small individuals (larvae or early juveniles) and high-water turbidity, which can compromise second-order metrics such as swimming speed and direction. These limitations could be mitigated with higher-resolution imaging systems, albeit at higher cost and computational requirements. Ongoing work focuses on refining and validating behavioral metrics and integrating the detection and tracking pipeline into a user-friendly graphical interface to facilitate its adoption in aquaculture and ecological research.

# PHYTOBIOTIC-ENRICHED AQUAFEED: HARNESSING EUCALYPTUS ESSENTIAL OIL TO ENHANCE HEALTH AND IMMUNITY IN JOUVENILE SEA BASS (*Dicentrarchus labrax*)

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## Introduction

Aquaculture, the fastest-growing production sector globally, provides essential protein and nutrients but faces productivity and sustainability challenges due to disease outbreaks in intensive systems, worsened by climate change (Liao et al., 2024). The excessive use of antibiotics has raised concerns about antimicrobial resistance and environmental safety, prompting the need for alternative approaches to health management (Reverter et al., 2021). Phytobiotics—natural bioactive compounds from plants—are emerging as effective immunostimulants, with *Eucalyptus globulus* essential oil showing notable antimicrobial and immune-enhancing effects in fish (Lin et al., 2025). This research aims to develop a novel functional aquafeed enriched with eucalyptus essential oil to enhance immune function in juvenile European seabass (*Dicentrarchus labrax*).

## Materials and Methods

Bioactive compounds with immunoenhancing properties in the essential oil were identified using GC-MS. Analysis was performed with a Shimadzu GC-2030 system and GCMS-QP2020 NX detector, using an SP-2340 capillary column. Helium was the carrier gas (1 mL/min, splitless mode). The oven temperature was programmed from 43 °C to 230 °C, then held for 12 min, followed by a post-run at 240 °C for 5 min. A 96-well plate microdilution method was used to evaluate the antibacterial activity of the extract against four marine pathogens: *Listonella (Vibrio) anguillarum* serotype O1, *Edwardsiella anguillarum*, *Vibrio harveyi*, and *Aeromonas veronii* biovar *sobria*. The extract was mixed with sterile BHIB at a 1:1 v/v ratio, and 10 µl of each bacterial culture was added. Plates were incubated at 25°C for 72 hrs. Optical density (OD) at 610 nm was measured at 12, 24, 48, and 72 hours using a TECAN Infinite® M Plex microplate reader. For the in vivo feeding trial, 200 juvenile *D. labrax* of 27.6 ± 0.1 g initial mean weight were randomly distributed into closed seawater circulation system tanks of 500 L capacity. The groups were fed two different isonitrogenous and isoenergetic diets. The Control diet was formulated to resemble a commercial diet for sea bass, without phytobiotic, and the experimental diet was formulated to contain 0.1% *E. globulus* essential oil. Fish were fed at a feeding rate of 1.5% of their body weight per day, six days per week for a total of 8 weeks. Water conditions were monitored daily: temperature (19–21°C), salinity (37± 0.2‰), pH (8.0 ± 0.1), and dissolved oxygen (>5.5 mg/ml).

## Results and Discussion

The resulting chromatogram is shown in Figure 1, illustrating the major constituents of the oil based on their retention times and relative intensities. The chemical composition of eucalyptus essential oil was determined via GC-MS analysis. The GC-MS analysis of eucalyptus essential oil identified eucalyptol (1,8-cineole) as the major component (68.9 ± 0.27%), with d-limonene (1.53%) and p-cymene (1.01%) as minor constituents. These results align with previous findings showing eucalyptol as the dominant compound in *E. globulus* oil. Its high eucalyptol content supports its potential use as a natural phytobiotic in aquaculture, offering antimicrobial and anti-inflammatory benefits that promote fish health. (Mączka et al., 2021; Lin et al., 2025).

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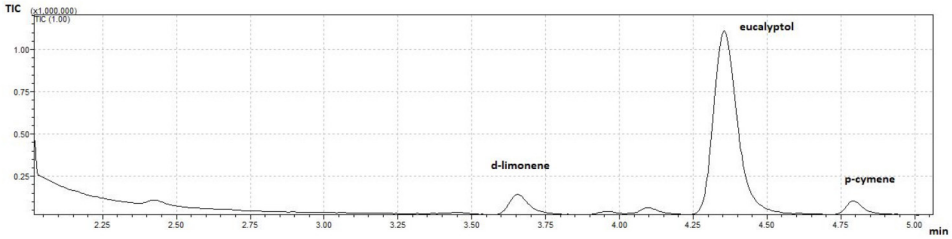
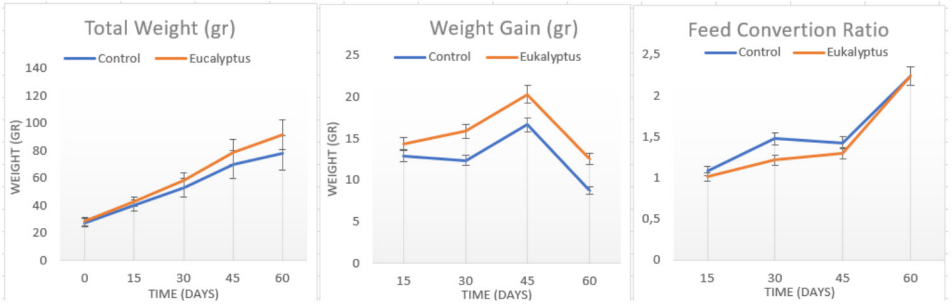



**Figure 1.** Representative chromatogram of eucalyptus essential oil obtained by GC-MS analysis.




The extract significantly inhibited the growth of all tested pathogens (*L. anguillarum* O1, *E. anguillarum*, *V. harveyi*, and *A. veronii* bv *sobria*) throughout the 3-day incubation. The supplemented feed was well accepted, with no mortalities or behavioral changes observed. Fish fed the supplemented diet showed significantly higher ($P<0.05$) total weight from the first month onward, with noticeable weight gain starting at day 15. The feed conversion ratio was lower for the experimental group, particularly during the first 45 days.

The enhanced growth performance that was observed along with the efficient feed utilization shown by the lower feed conversion ratio is a possible indicator for an enhanced gut health and overall good fish health condition provided by the feed supplementation.

**Acknowledgements**

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# EVALUATING THE DIGESTIBILITY OF BREWER'S SPENT GRAIN PROTEIN ISOLATE FOR THE RAINBOW TROUT *Oncorhynchus Mykiss* DIET

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## Introduction

Aquafeed, as major input in aquaculture still relies upon conventional plant protein source like soy. However, the use of soy in aquafeeds are associated with ecological concerns, price volatility, and reduces its availability for direct human consumption (Wang et al., 2023). Brewers spent grain (BSG) is a major agro-industrial byproduct and a potential alternative protein source for soy with the annual production of 40 million tonnes. However, BSG utilization in aquafeed in its raw form is limited due to very low digestibility associated with high fibre content (Karlsen & Skov, 2022). These limitations can be overcome by extracting BSG protein isolate (PI), leaving behind fibre and other anti-nutritional compounds. Evaluating the digestibility of extracted BSG PI is essential to ensure its nutritional viability. In this study, both *in vitro* and *in vivo* digestibility of BSG PI are determined for rainbow trout in comparison with soybean meal to validate the BSG PI as a potential alternative protein source in rainbow diet.

## Materials and methods

BSG and protein extraction

Raw BSG was procured from San Miguel Mahou Brewery Ltd., Lleida. Protein extraction was carried out in pilot food industry of IRTA, Monells. Initially, wet BSG biomass was mixed with water at 1:4 (w/w) and heated to 50 $^{0}$C. Protein extraction was carried out at pH 11.5 using 2M NaOH 2 hours with constant stirring. The slurry was decanted to separate the liquid phase and reduced the pH to iso-electric point (3.8) The precipitated protein was separated using industrial centrifuge and washed with water to remove excess solutes and neutralized to pH 7 before freeze drying.

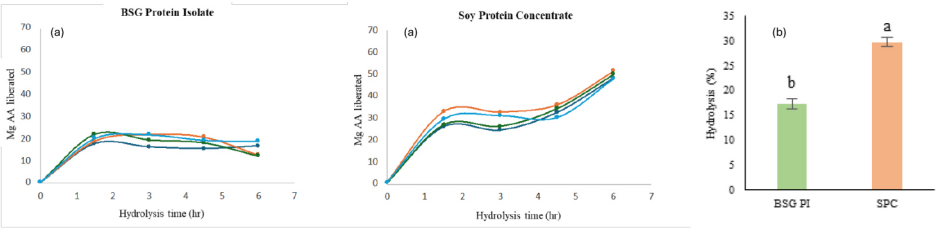


*Figure 1. Dynamics of amino acids liberation during in vitro digestion (a) and protein hydrolysis values (mean ± SD) of the ingredients evaluated (b).*

*Table 1. Apparent Digestibility Coefficient (ADC) of dry matter, protein, and energy of the experimental diets, and growth and feed efficiency of rainbow trout (O. mykiss) fed diets.*

| Treatment | BG0 | BG15 | BG25 | BG50 | BG100 | ANOVA |
|---|---|---|---|---|---|---|
| $ADC_{DM}$ (%) | 77.03 ± 0.80 | 76.63 ± 0.90 | 75.93 ± 1.47 | 78.26 ± 1.16 | 76.93 ± 1.69 | 0.096 |
| $ADC_{Protein}$ (%) | $94.47^{b}$ ± 0.22 | $94.16^{b}$ ± 0.08 | $93.70^{ab}$ ± 0.27 | $94.12^{b}$ ± 0.65 | $93.34^{a}$ ± 0.55 | 0.008 |
| $ADC_{Energy}$ (%) | 82.86 ± 0.53 | 82.84 ± 0.84 | 82.42 ± 1.31 | 83.63 ± 0.64 | 82.66 ± 0.89 | 0.300 |
| Final BW (g) | $214.59^{b}$ ± 4.68 | $209.09^{ab}$ ± 5.36 | $212.34^{b}$ ± 2.96 | $207.19^{ab}$ ± 5.32 | $202.88^{a}$ ± 2.67 | 0.004 |
| SGR(% $day^{-1}$) | $2.06^{b}$ ± 0.04 | $2.01^{ab}$ ± 0.05 | $2.04^{b}$ ± 0.02 | $2.00^{ab}$ ± 0.04 | $1.96^{a}$ ± 0.02 | 0.004 |
| FCR | $1.00^{a}$ ± 0.04 | $1.03^{ab}$ ± 0.02 | $1.00^{a}$ ± 0.02 | $1.03^{ab}$ ± 0.04 | $1.06^{b}$ ± 0.02 | 0.030 |

*(Continued on next page)*

*In vitro* and *in vivo* Digestibility

For *in vitro* assay, the stomach and intestine sections of rainbow trout (350 g BW) after 6 hours post-ingestion were used to prepare functional extracts. The acid and alkaline phases of protein hydrolysis was simulated in a two-chambered bioreactor separated by a semi-permeable dialysis membrane with a molecular weight cut of 3,500 Da as described by Lewis et al. (2019). Measurement of acid and alkaline protease by tyrosine release from hydrolysis of haemoglobin (pH 2.5) (Anson 1938) and casein (pH 8.5) (Walter, 1984) respectively. The duration of the acid and alkaline phases of digestion were 1.5 and 4.5 hours respectively. Samples of BSG protein isolates (58.7% CP) and soy protein concentrate, SPC (61.5% CP) were used for comparison. All assays were performed in quadruplicate. *In vivo* assay is conducted as part of ongoing replacement trial on rainbow trout. Five diets were prepared by replacing gradient levels of SPC using BSG PI with the addition of inert marker. The in vivo digestibility will be analysed by measuring the ratio of nutrients and marker in the diet and faecal matter.

**Results and discussion**

The dynamics of amino acid release during protein hydrolysis of BSG PI and SPC is detailed in Figure 1.

Figure 1 shows hydrolysis profiles of SPC and BSG PI that are generally characterized by a strong initial effect of acid hydrolysis, followed by a stable phase at the beginning of the alkaline phase and a second increase in the release of amino acids after 3 h of alkaline digestion. However, the hydrolysis profile of the BSG PI was clearly different in both acid alkaline digestion phases. It showed slightly lower values than SPC during the acid digestion phase. But the drastic effect of hydrolysis was observed at alkaline phase without any increase in amino acid liberation after 3 hours of alkaline digestion.

The final hydrolysis values of BSG PI and SPC after *in vitro* digestion with rainbow trout digestive enzyme extract were presented in Figure 2. Overall, final hydrolysis value of BSG PI (17.3% ± 1.05) was significantly lower ($P<0.05$) compared to SPC (29.78% ± 0.84). It is difficult to directly predict the actual *in vivo* digestibility of the BSG PI via *in vitro* hydrolysis. Because various factors play role in a digestibility in the live organism such as interactions with feed matrix, actual digestion duration, effect of intestinal microbiota etc (Moyano et al., 2015). This is especially obvious in ingredients containing complex polysaccharides. Lower *in vitro* digestibility of BSG PI in the present study will be validated in *in vivo* by an ongoing feeding trial in terms of key performance indicators associated to growth performance, feed efficiency.

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# MODULATION OF GONAD DEVELOPMENT, OXIDATIVE STRESS AND GENE EXPRESSION THROUGH CAROTENOID PROVISION IN *Paracentrotus lividus* (LAMARCK, 1816) UNDER HIGH TEMPERATURE

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## Introduction

Sea urchin populations have dramatically declined due to continuous alterations in ocean conditions caused by global climate change. Elevated temperatures increase the energetic cost of maintaining essential physiological functions and consequently disrupt defense mechanisms against reactive oxygen species (ROS) in marine invertebrates. This leads to oxidative stress and lipid peroxidation, ultimately reducing their fitness and increasing their susceptibility to mortality (Leiva et al., 2022). Recent research highlights that sea urchins can alter their physiological responses through phenotypic plasticity after acclimation. Dietary carotenoids can help counteract oxidative damage, protecting cellular components and potentially improving the efficiency of the immune response and reproductive success. Major yolk protein (MYP) is the nutritional reserve in oocytes, and it is essential for embryonic development. Temperature-induced reproductive disruptions inevitably affect the availability and function of MYP. Conversely, heat shock proteins (HSPs) are activated under thermal stress, act as molecular chaperones to protect and refold denatured proteins, and play an important role in thermal adaptation. Understanding the interplay between these factors is crucial for developing effective strategies for the sustainable production and conservation of sea urchins in a changing environment. The present study aimed at evaluating the impact of high and low carotenoid diets on the reproductive performance, oxidative stress, and gene expression of *myp* and heat shock proteins (*hsp70, hsp90, hsp60*, and *hsp56*) in the sea urchin *Paracentrotus lividus* (Lamarck, 1816) under high temperature.

## Materials and Methods

*P. lividus* broodstock was acclimated in recirculating aquaculture systems (RAS) and starved for one month before the experimental conditions. Sea urchins (N=168) were reared for four months on two temperature scenarios: local environmental temperature (18°C) and warming temperature (+3.5°C), following IPCC 2023 predictions for 2100. During conditioning, the individuals were fed with two isoproteic and isolipidic jellified diets: a high carotenoid diet (HC) and a low carotenoid diet (LC). The microalgae *Nannochloropsis* sp. was used as a source of carotenoids since it enhances gonad development and immune function **(**Tsushima et al., 1991**).** During acclimation, a 12L:12D photoperiod was used to mimic natural light conditions. The temperature was digitally controlled, simulating the two scenarios. By the end of the trial, several physiological parameters were assessed, including maturity level, gonadosomatic index (GSI), and oxidative stress markers such as superoxide dismutase (SOD) and catalase (CAT) activity, as well as lipid peroxidation (LPO). Subsequently, total RNA was extracted from gonad tissue and used to perform RT-qPCR for gene expression analysis of *myp* and the heat shock protein genes *hsp70, hsp90, hsp60*, and *hsp56*.

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**Results**

Conditioning temperature significantly influenced gonad development. Sea urchins maintained at +3.5ºC and fed with both diets presented a higher proportion of advanced stages of gonad development (mature; partly spawned; spent). GSI was significantly affected by temperature, with lower values observed in sea urchins exposed to +3.5°C and fed the HC diet (7 %). The activities of antioxidant enzymes (SOD and CAT) and the level of LPO were affected by temperature and dietary treatments. SOD activity showed a decrease of over 10% when sea urchins were exposed to +3.5°C and fed with both diets, with no significant differences observed between the HC and LC diets at this temperature. In contrast, CAT activity significantly increased (39 Umg/protein) in sea urchins fed the LC diet and exposed to +3.5°C, compared to those kept at 18°C (11 Umg/protein). When the sea urchins were fed by the HC diet, no significant differences in CAT activity were observed between the two temperature treatments, with activity levels remaining relatively stable (between 10-16 Umg/protein). A similar trend was observed for LPO. Significantly higher LPO levels (0.05 nmol/g wet weight) were detected in sea urchins exposed to +3.5°C and fed the LC diet. The expression of *myp* was significantly up regulated in sea urchins exposed to the +3.5°C temperature, regardless of the dietary treatment. Conversely, the expression of heat shock protein genes (*hsp70*, *hsp90*, *hsp60*, and *hsp56*) increased in sea urchins exposed to +3.5°C, particularly in those fed the LC diet.

**Discussion**

Sea urchins fed the HC diet demonstrated the strongest antioxidant defense when exposed to a higher temperature. These individuals exhibited high SOD concentration and significantly reduced CAT activity, resulting in low LPO values. Tsushima et al. (1991) reported that the carotenoid supplementation in a diet improves the immune function of sea urchins. Although CAT activity was higher when sea urchins were exposed to a higher temperature and fed with LC diet, it was insufficient to prevent lipid peroxidation, resulting in the highest LPO values. The expression of the heat shock protein genes was up regulated when the individuals were exposed to higher temperatures and fed with an LC diet. The combination of high temperature and a low carotenoid diet creates a synergistic stress effect. The high temperature directly induces protein misfolding and ROS production, while the low carotenoid diet weakens the sea urchins' ability to counteract the resulting oxidative stress. The up regulation of *myp* gene expression indicates that sea urchins were responding to higher temperatures by enhancing their reproductive processes, specifically egg development. The increase of nutrient accumulation in the gonads could be a mechanism to increase the offspring's resilience and maximize the reproductive success in a changing environment.

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# EVALUATION OF AEROBIC MINERALIZATION AS A TREATMENT FOR EFFLUENTS FROM AN AQUAPONIC SYSTEM

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Aquaponics is a sustainable production system that integrated techniques from both hydroponics and aquaculture. Although aquaponic systems are water-efficient, the cleaning of the clarifier leads to the loss of essential plant nutrients. Therefore, the mineralization of aquaponic effluents has been increasingly studied as a strategy to recover both water and nutrients. In this context, implementing mineralization processes in aquaponics allows the transformation of solid wastes into inorganic compounds that are highly available and efficient for plant growth. This study aimed to evaluate aerobic mineralization as a treatment for effluents from an aquaponic system by determining nutrient dynamics and water quality parameters. Two treatments were evaluated, each with five replicates: aerobic mineralization using filtered and unfiltered water collected from the clarifier of an aquaponic system with different lettuce varieties and Nile tilapia. Water samples were collected over a one-month period on days 0, 7, 15, 23, and 30. The following nutrients and water quality parameters were analyzed: ammonium, nitrite, nitrate, iron, phosphate, calcium, potassium, manganese, magnesium, molybdenum, zinc, sulfate, copper, total hardness, temperature (°C), pH, dissolved oxygen (% and ppm), turbidity (FNU), conductivity (µS/cm), and total dissolved solids. The results showed significant differences in macro- and micronutrient dynamics between the filtered and unfiltered treatments during mineralization. Between the days 15 and 23, an increase in pH from acidic to alkaline values was recorded, enhancing nutrient solubility and mineralization rates. Nitrogen mineralization showed efficient conversion from ammonium to nitrite and subsequently to nitrate, the preferred nitrogen form for plant absorption. Phosphate levels initially increased under acidic conditions but decreased as pH became alkaline. Magnesium and molybdenum concentrations were higher at low pH, while calcium, potassium, zinc, and total hardness increased under neutral to alkaline conditions. Aerobic mineralization with filtered water resulted in improved nutrient concentrations and higher nitrification rates, leading to greater nitrate availability. This study highlights that the implementation of aerobic mineralization processes in aquaponic wastewater management enhances nutrient transformation and recovery, simultaneously improving water use efficiency. CIAS 4164.

# EFFECT OF BIOFLOC CONSERVATION ON BACTERIAL POPULATIONS AND EVALUATION OF THEIR EVOLUTION FOLLOWING BIOFLOC REINOCULATION

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## Introduction

Biofloc technology (BFT) is a proven production system that enables the cultivation of aquatic animals at high densities while maintaining water quality through the activity of various organisms, mainly bacteria. These bacteria perform multiple key functions, including nutrient transformation, nitrogen compound removal, floc formation, and organic matter exchange. Starting a biofloc system requires time and can lead to ammonium and nitrite peaks in the water, but reinoculation with mature biofloc can accelerate the process, provided that an established system is available. Otherwise, preserving biofloc may offer a solution to reduce maturation time, as long as its microbial and functional diversity is maintained during storage.

## Materials and methods

Mature biofloc was collected, decanted and filtered to reduce moisture content. Afterwards, samples were subjected to three preservation treatments: refrigerated vacuum (RV), frozen (F), and dry (D). RV samples were stored in vacuum-sealed bags at 4°C. F samples were mixed with 15% glycerol and stored at -80°C. D samples were dried at 40°C. A negative control (NC) was not inoculated, while a positive control (PC) was directly inoculated with fresh, decanted, and pressed biofloc. All inoculated groups received biofloc at a concentration of 1 g/L. These preservation techniques are patented (*P202530281*). The storage period before inoculation was 7 days. Each treatment had three replicates (80 L tanks, aerated, at 28°C). *Penaeus vannamei* (76 g/tank) were stocked. Water samples were collected weekly to analyze nitrogen compounds (ammonium, nitrite, nitrate) and phosphorus (phosphate) concentrations. Microbial diversity was analyzed on days 0, 6, 21, and 41.

The full-length 16S rRNA gene was amplified using barcoded primers 27F and 1492R. PCR (27 cycles) was followed by quality control and equimolar pooling. Libraries were prepared with the SMRTbell Express Template Prep Kit 2.0 and sequenced on a PacBio Sequel II. Sequences were processed with QIIME2 using DADA2 for denoising. Taxonomy was assigned with the SILVA 138 database. Alpha diversity (Shannon, Simpson, Chao1) and beta diversity (Bray-Curtis, PCoA) were calculated. Differential abundance was analyzed with ALDEx2; Krona plots visualized taxonomic profiles.

| | Ammonium-N (mg N/L) | | | Nitrite-N (mg N/L) | | |
|---|---|---|---|---|---|---|
| | Max ± SE | Min ± SE | Mean ± SE | Max ± SE | Min ± SE | Mean ± SE |
| Negative Control (NC) | 4,08±0,58 **b** | 0,01±0,01 | 0,94±0,35 **b** | 13,63±0,22 **c** | 0,00±0,00 **a** | 3,27±1,09 **b** |
| Refrigerated Vacuum (RV) | 0,07±0,00 **a** | 0,00±0,00 | 0,04±0,01 **a** | 0,05±0,01 **a** | 0,00±0,00 **a** | 0,06±0,02 **a** |
| Frozen (F) | 0,01±0,01 **a** | 0,00±0,00 | 0,06±0,01 **a** | 0,23±0,05 **a** | 0,00±0,00 **a** | 0,06±0,01 **a** |
| Dry (D) | 3,36±0,40 **b** | 0,00±0,00 | 0,59±0,26 **ab** | 7,86±0,37 **b** | 0,03±0,01 **b** | 2,52±0,83 **b** |
| Positive Control (PC) | 0,11±0,01 **a** | 0,00±0,00 | 0,03±0,01 **a** | 0,12±0,04 **a** | 0,00±0,00 **a** | 0,05±0,02 **a** |

*Table 1. Maximum, minimum, and mean concentrations (± SE) of Ammonium-N and Nitrite-N under different biofloc preservation treatments.*

| | Nitrate-N (mg N/L) | | | Phosphate-P (mg P/L) | | |
|---|---|---|---|---|---|---|
| | Max ± SE | Min ± SE | Mean ± SE | Max ± SE | Min ± SE | Mean ± SE |
| Negative Control (NC) | 44,93±3,32 | 2,11±1,11 **ab** | 17,66±3,64 | 2,36±0,10 **b** | 0,06±0,00 **c** | 0,92±0,19 **b** |
| Refrigerated Vacuum (RV) | 55,22±3,20 | 4,16±0,40 **b** | 21,37±3,80 | 0,20±0,09 **a** | 0,04±0,01 **b** | 0,87±0,18 **a** |
| Frozen (F) | 47,80±4,44 | 1,31±0,21 **a** | 26,95±3,74 | 1,48±0,61 **b** | 0,00±0,00 **a** | 1,11±0,13 **b** |
| Dry (D) | 45,75±1,80 | 2,51±0,14 **ab** | 19,11±3,88 | 2,49±0,06 **b** | 0,30±0,01 **e** | 1,25±0,20 **b** |
| Positive Control (PC) | 53,28±4,23 | 2,88±0,62 **ab** | 26,25±3,89 | 1,38±0,28 **b** | 0,19±0,00 **d** | 0,13±0,02 **b** |

*Table 2. Maximum, minimum, and mean concentrations (± SE) of Nitrate-N and Phosphate-P under different biofloc preservation treatments.*

*(Continued on next page)*

## Results and discussion

After 41 days, no Ammonium-N and Nitrite-N peaks were observed in RV, F, and PC, indicating effective nitrogen removal (Table 1). Nitrate-N levels did not differ between PC and preserved biofloc (RV, F, D), suggesting reinoculation did not increase nitrate accumulation (Table 2). Phosphate-P was significantly lower in RV than in other treatments, likely due to microbial assimilation, as no water exchanges were made and only dissolved phosphate was measured (Table 2). These results suggest that refrigeration and freezing preserve microbial activity related to nitrogen and phosphorus cycling.

| | Inicial day | | | |
|---|---|---|---|---|
| | **Shannon** | **Simpson** | **OBS** | **CHAO1** |
| **Negative Control (NC)** | 0,31±0,18 **a** | 0,18±0,13 **a** | 1,25±0,19 **a** | 2,50±0,50 **a** |
| **Refrigerated Vacuum (RV)** | 1,78±0,02 **c** | 0,78±0,00 **b** | 4,54±0,06 **b** | 25,00±1,73 **b** |
| **Frozen (F)** | 1,39±0,04 **b** | 0,65±0,01 **b** | 2,92±0,06 **b** | 23,00±1,00 **b** |
| **Dry (D)** | 0,22±0,12 **a** | 0,09±0,06 **a** | 1,10±0,07 **a** | 6,00±3,46 **a** |
| **Positive Control (PC)** | 1,85±0,05 **c** | 0,77±0,01 **b** | 4,33±0,09 **b** | 27,33±1,53 **b** |

*Table 1. Alpha diversity at the phylum level for each treatment on initial and final days. Values represent mean ± SE. Different letters indicate significant differences ($p < 0.05$).*

At day 0, alpha diversity at the phylum level (Shannon, Simpson, Chao1, OBS) was significantly lower in NC and D, indicating reduced microbial richness compared to RV, F, and PC. RV and PC were similar, showing that refrigeration effectively preserved phylum-level diversity, while F showed a moderate reduction. After 41 days, all diversity indices converged across treatments, suggesting that biofloc maturation stabilized microbial communities at the phylum level.

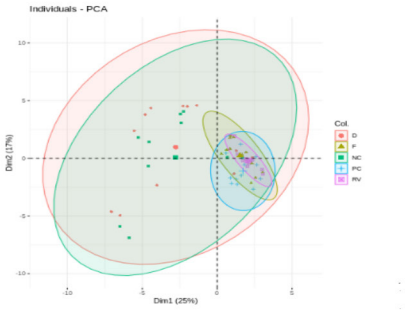


*Figure 1.Principal Component Analysis (PCoA) of microbial communities at the*

Beta diversity at the phylum level, visualized by PCA (Figure 1), showed that RV and PC communities clustered closely, indicating similar microbial structures. F samples were moderately similar, while NC and D formed separate and more dispersed clusters, reflecting higher variability due to lack of inoculation or drying. RV, F, and PC showed lower dispersion, likely due to better preservation of the initial community.

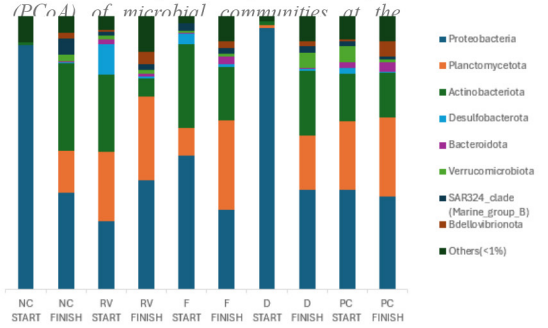


*Figure 2. Relative abundance of bacterial phyla in each treatment at the start and after 41 days of biofloc maturation.*

Respect to taxonomy compositions, Proteobacteria and Planctomycetota were the most dominant phyla across all treatments (Figure 2). Significant differences were observed between the initial communities of NC and RV, particularly in these two phyla, suggesting that refrigeration preserved a more functional microbial structure. In contrast, no significant differences were found between the initial profiles of RV and PC, indicating effective preservation of the original biofloc community in RV. For F treatment, similar patterns were observed; however, significant differences emerged between the initial F and PC communities, suggesting a partial alteration due to freezing. Notably, nitrifying bacteria, which are crucial for nitrogen cycling, belong to the Proteobacteria phylum, highlighting the importance of maintaining this group during biofloc preservation.

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# ENHANCED IMMUNE RESPONSE IN RAINBOW TROUT BY ORAL VACCINATION WITH MICROALGAE-EXPRESSED IHNV GLYCOPROTEIN

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Introduction

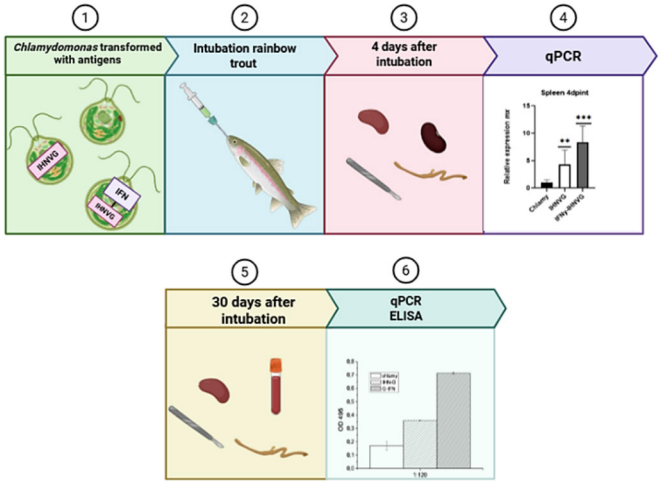
Infectious hematopoietic necrosis virus (IHNV, Salmonid novirhabdovirus) poses a major threat to salmon and trout aquaculture around the globe, with the virus nowadays present in America, Asia and Europe (1). Significant efforts have been made to develop an effective vaccine to prevent the spread of the disease. Most of the experimental anti-IHNV vaccines reported so far are inactivated virus or DNA vaccines, usually administered by injection (2). The protective efficacy of injected vaccines has been fairly good in most cases, with one licensed vaccine (Apex IHNV, Canada) available. In contrast, the development vaccines designed for oral administration to fish (a most desirable way to be implemented in aquaculture facilities) has been challenging (3).

Previous studies by our group demonstrated the efficacy of recombinant vaccines based on the expression of the main antigenic region of the rhabdoviral glycoprotein (G) in E. coli (as nanopellets) against spring viremia of carp virus (SVCV) in zebrafish and viral hemorrhagic septicemia virus (VHSV) in rainbow trout (4).

In the present work, a fragment of the IHNV glycoprotein (G) alone as well as fused to gamma interferon were expressed in the microalga *Chlamydomonas reinhardtii*. The final goal of the project is to deliver an oral vaccine to be used in rainbow trout aquaculture as a preventive method to control IHNV outbreaks.

Results

A 190-mer fragment of IHNV G protein was successfully cloned into a *Chlamydomonas reinhardtii* expression vector, as well as the IHNV-G fused to trout gamma interferon. Positive clones were selected by growing on antibody-agar plates. Next, the production of the antigens (IHNV-G, IFN-IHNG) was verified by western-blot analysis, observing the bands at the expected size (26,1kDa and 45kDa, respectively).

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Cultures of clones expressing IHNV-G and IFN-IHNG were concentrated and lyophilized. After reconstitution in PBS the viral antigens were delivered to rainbow trout fingerlings (three 6mg doses per fish) by intragastric gavage. At 4 and 30 days post-intubation, serum, head kidney, spleen and midgut samples were collected for analysis. When using IFN-IHNG as antigen ELISA OD values in sera were significantly higher at 30 days post intubation than in the control group (fish immunized with non-transformed microalgae). The IHNV-G group showed relatively higher OD values than controls, but the difference was not significant.

Immune-related genes expression was analyzed in the mid gut, spleen and head kidney at three time points after intubation. At early time (4 dp.int.), two interferon-inducible antiviral protein genes (*mx* and *vig*) were significantly augmented in head kidney, spleen and intestine of fish intubated with IFN-IHNG. At later times (30 days) up-regulation of *mx* and *vig* genes in spleen and intestine was observed. Immunoglobulin M (*IgM*) gene expression was not found significantly changed at any time point in immunized fish.

In a follow up experiment, the efficacy of the microalgae-based oral vaccine will be tested on rainbow trout under a diet supplemented with recombinant *Chlamydomonas* and subsequently challenged with IHNV.

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# ANTIVIRAL ACTIVITY OF ALGAE EXTRACTS FROM *Chlamydomonas reinhardtii* AGAINST SPRING VIREMIA OF CARP VIRUS

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Aquaculture is a growing productive sector that is frequently threatened by viral diseases, particularly those caused by rhabdoviruses such as the spring viremia of carp virus (SVCV). The limited availability of effective antiviral treatments has encouraged the search for natural alternatives, including the use of microalgae as sources of bioactive compounds. This study evaluates the antiviral effect of aqueous and methanolic extracts from the green microalga *Chlamydomonas reinhardtii*, against SVCV in vitro. Exposure to the extract was performed as pretreatment of the cells before infection, as well as coincubation with the extract during viral entry. The Chlamydomonas methanolic extract displayed a significant inhibitory activity on the virus cytopathic effect, reaching 89% protection in cells preincubated with the extract before SVCV infection, whereas the aqueous extract showed negligible antiviral activity. Viral RNA levels also decreased after exposure to the extract. Interestingly, incubation of the cells with the extract enhanced interferon and mx immune responses. Based on our findings the Chlamydomonas methanolic extract can be considered a promising natural therapeutic agent for mitigating SVCV pathogenicity. Additionally, this may pave the way for future research microalgae as dietary supplements for fish, aiming to prevent viral infections in a safe and sustainable manner.

**Acknowledgements**
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# KEY NUTRIENTS IN FISH PROTEIN-BASED PRODUCTS BEHIND THE GROWTH RESPONSE, SKELETAL DEVELOPMENT AND GASTRO-INTESTINAL ENZYME ACTIVITIES OF EUROPEAN SEABASS *Dicentrarchus labrax* FRY

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## Introduction

Fish protein hydrolysates (FPH) are products from either chemical or enzymatic breakdown of fish raw material into single amino acids, peptides and oligopeptides. They are considered a suitable source of protein for animal nutrition because of their balanced amino acid composition and their low molecular weight, allowing higher gut absorption rates and functional properties. The inclusion of FPH in the range of 5-40% in diets has been reported to increase survival and growth rate, decrease malformation rate, increase enzyme activity, modify nutrient transport patterns in the intestine and induce non-specific immune responses in larvae, fry and adult fish. The positive effects of dietary FPH inclusion at the earlier stages of development are related to the fact that the gastrointestinal tract (GIT) is still under development; therefore, providing the fish with medium and small-size peptides through the diet instead of with intact proteins may help in the process of absorption through the enterocytes. Traditionally, this type of studies has been conducted by replacing certain levels of the dietary fishmeal (FM) with similar levels of FPH either as a fraction of the diet or as a fraction of the dietary protein contribution. In most cases, results have been explained based on the final dietary amino acid composition and/or the water-soluble peptides fractions distributions; however, less attention has been given to other nutrients provided by different fish products, which also vary with the dietary source exchange and may play a significant role in the evaluated responses. The present activity aimed to identify key nutrients responsible for variations in the growth rates, the development of haemal lordosis and GIT enzyme activities of E. seabass fry (0.2 – 6 g) fed with two different levels of dietary FM (53% and 65%) and FPH (0% and 13%).

## Materials and methods

Fingerlings of E. seabass (0.2 g body weight) were fed with the 4 experimental diets, in triplicate groups, for 70 days. When fish reached approximately 1.5 g body weight, they were subjected to a swimming challenge test by forcing them to swim against a water current of approximately 17-18 cm/sec for 7 days. Before and after this challenge, samples of fish were taken to document the development of haemal lordosis. At the end of the 70-d feeding trial, and following a recovery period, samples of fish were taken for analyses of growth performance, GIT enzyme activities and haemal lordosis. Data were analysed by means of 2-way ANOVA. In addition, Principal Component Analyses (PCA) were performed to identify potential correlations between the response variables and the dietary nutrients composition.

## Results and conclusions

In general, the growth of the fish was not significantly affected by dietary treatments at the end of the 70-d feeding period. However, GIT enzymes such as trypsin, pepsin, leucine aminopeptidase (LAP) and lipases did respond differently to treatments. Similarly, we found a significant interaction between dietary FM and FPH levels in the development of haemal lordosis and observed a variation in their levels before and after the swimming challenge test. As expected, the amino acid and peptides distribution profiles are key responsible for the observed parameters; however, the potential role of minerals, trace elements, lipo-soluble vitamins and phospholipids are also defined.

# BIOFOULING CONTROL, PLASTIC FOOTPRINTS AND FISH WELLFARE: EVALUATING NET MATERIALS AND CLEANING METHODS IN MARINE FARMS

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Aquaculture relies extensively on polymer-based infrastructure and equipment, which likely contributes to microplastic (MP; <5 mm) release into the marine environment. Beyond feed pipes and cage collars, net pens represent a notable source of MPs. In situ net cleaning—typically performed with in situ intermittent cleaning using high pressure washers—can intensify abrasion of net fibers and coatings, thereby increasing MP emissions during cleaning operations. Consequently, both synthetic fibers and particles derived from protective coatings may be dispersed into surrounding seawater. Addressing this emerging concern, the No-Gro project sought to improve biofouling management on fish-farming nets by systematically evaluating cleaning technologies with respect to fish health, environmental sustainability, and operational costs.

In this study, the Remora Robotics AS autonomous operated cleaning vehicle equipped with brush-based cleaning interfaces, is systematically compared to conventional pressure washing methods, evaluating performance, efficiency, and the release of microplastics environmental impact across both field deployments in real scenario and controlled laboratory settings designed to isolate key variables and replicate typical cleaning scenarios. The project assessed MP emissions using a combined approach of field monitoring and controlled laboratory abrasion tests, structured within elements of a safer-and-sustainable-by-design (SSbD) framework. In the field, routine cleaning events were monitored to quantify MPs, evaluate cleaning effectiveness, and track potential implications for fish welfare. In the laboratory, abrasion experiments simulated cleaning-induced wear to quantify and compare MP release from conventional multifilament nylon nets and alternative monofilament high-density polyethylene (HDPE) nets. Tests compared automated net-cleaning vehicles (AOVs) with pressure-washing technology using aluminium-framed 80 × 80 cm net panels submerged in a 300 L tank (fig.1). Released particles were recovered by filtration (≥10 $\mu$m) and quantified and characterized by Fourier-transform infrared (FTIR) microscopy imaging.

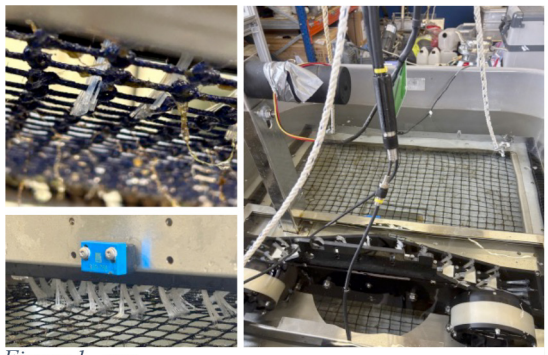

*Figure 1 - xxx*

In the filed study, areas where cleaning technologies operated showed higher levels only for polyethylene and polypropylene, reflecting the material makeup of cleaning equipment and its association with other aquaculture activities ie., HDPE pipes operated fish feed supply. The remaining detected polymer types where homogeneously distributed across all investigated sites. The results of the laboratory observations demonstrated clear material-dependent emission patterns: multifilament nylon nets released substantially more MPs than monofilament HDPE, underscoring the higher susceptibility of multifilament structures to mechanical wear. Field campaigns during routine cleaning captured sporadic and highly variable MP signals from polyethylene and polypropylene polymers, influenced by, fouling levels, and local hydrodynamic conditions. Together, these findings indicate that MP emissions are governed by polymer composition, filament architecture and the choice of cleaning technology. In laboratory-based studies, responses of material type and applied cleaning technology to microplastic emissions were observed. Independent of the cleaning technology employed, nylon nets exhibited the greatest microplastic emissions, with pressure cleaning releasing larger and more elongated fragments compared with brushing. In simulations of HDPE net cleaning, brushing technology produced a greater release of microplastic fragments than did the pressure washing solution. By adopting design-informed material selection and optimizing cleaning strategies, aquaculture operations can reduce MP releases at source while maintaining operational performance. The SSbD-oriented evidence generated by No-Gro provides actionable guidance for transitioning toward cleaner, more efficient, and environmentally responsible net-cleaning practices in marine aquaculture.

# A GUT INFLAMMATORY MODEL FOR AQUACULTURE NUTRITION

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**Introduction**

The health and functionality of gastrointestinal tract are fundamental to fish welfare, growth performance, and feed efficiency. Among the key challenges affecting gut health is inflammation—a physiological response triggered by either internal and external stressors such as the presence of anti-nutritional factors, pathogenic infections, and environmental stressors. Inflammatory conditions in the gut can disrupt nutrient absorption, impair immune responses, and increase susceptibility to pathogens, ultimately compromising productivity and sustainability in aquaculture systems. Understanding the interaction between diet and gut health has long been a major endeavor in aquaculture nutrition research. Nutritional strategies that support intestinal integrity and modulate immune responses are being actively explored. Functional feed additives—including prebiotics, probiotics, phytogenics, algae-derived compounds, and immunostimulants—are being tested for their ability to prevent or mitigate inflammation and promote a balanced gut homeostasis.

Here we introduce a standardized experimental gut inflammation model for European seabass (*Dicentrarchus labrax*), induced by dextran sodium sulphate (DSS), for evaluating the functional properties of novel ingredients under controlled conditions. This model allow researchers to study the progression of inflammation at the histological, molecular, and biochemical levels, unraveling how different dietary components influence the gut performance landscape and consequent fish thrive.

**Material and Methods**

The gut inflammatory model was developed following concept trial where increasing dosages of dextran sodium sulphate (DSS) were included in a formulated feed, complying with juvenile European seabass nutritional requirements. In triplicate tanks, fish (aprox 70g body weight) were fed DSS-spiked diets for 15 days. For this time course, feed intake was monitored to ensure a standardised ingestion of the inflammatory agent (DSS). Intestinal responses were monitored through histological assessment of the gut epithelial cell architecture and conformation (gut villi length, goblet cells' mapping, lymphocytes infiltration). More, evaluation of the relative expression of a gene's panel related with inflammatory response (e.g. *IL1-β*, *IL-8*, *TNFα*), epithelium integrity (e.g. *TJP1*, *OCL*, *CLDN15*, *iMUC*) and osmoregulation (e.g. *AQP1*, *NKCC*) was performed by RT-qPCR normalizing for *β-actin*. In addition, inflammatory markers in the faeces (e.g. calprotectin and mucins) were quantified using standardized procedures for the species. After this controlled "inflammatory" phase , fish were fed the control diet and were monitored to assess the intestinal regenerative process.

**Results and Discussion**

Fish presented a good and stable feeding behaviour throughout the feeding period, even at highest dosage. Histological score – with higher scores indicating epithelium architecture damage – presented a positive correlation with the dosage of DSS, right after the 5$^{th}$ day of feeding, indicating damage of intestinal lining. From day 10 lymphocytes' infiltration was evident with increasing severity with DSS dosage. Molecular inflammatory responses were triggered after 5 days, with higher expression of *IL-8* and *TNFα*. On the other hand, at longer periods, the expression of epithelium related markers (*OCL*, *iMUC*) was reduced, indicating epithelium damage. The correlation between DSS dosage and intestinal inflammation was established and further validated in different trials. This standardized mild-inflammatory challenge and the associated diagnostic markers allow a precise monitoring of the inflammation progress, and its recovery, allowing a reliable assessment of novel ingredients and additives with protective or regenerative potential at the intestinal level.

# EXPLORING MUCOSAL RESILIENCE IN ATLANTIC SALMON: THE IMPACT OF NOVEL ALGAE-BASED FUNCTIONAL DIETS

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**Introduction**

The maintenance of optimal health and resilience in aquaculture is paramount for the achievement of sustainable production. The mucosal surfaces of the gastrointestinal tract, gills, and skin serve as the principal interfaces between the fish and its external environment, functioning as the initial line of defense against pathogens and various stressors. These mucosal systems—comprising the gut-associated lymphoid tissue (GALT), gill-associated lymphoid tissue (GIALT), and skin-associated lymphoid tissue (SALT)—are indispensable for host immunity, nutrient assimilation, and osmoregulation. Despite contemporary aquaculture practices that strive to mitigate stressors, Atlantic salmon are frequently exposed to adverse conditions that may jeopardize mucosal integrity and functionality, thereby diminishing their ability to endure disease and recover from various insults. Dietary interventions that support and modulate mucosal immunity offer a promising strategy to enhance fish health and welfare. Algae, both micro- and macroalgae, are valuable sources of bioactive compounds applied by nutraceutical and cosmeceutical industries, promoting tissue healing and regenerative capacity. In this study the effects of diets supplemented with various types of algae biomasses and extracts were assessed with the objective to determine how these dietary additions can enhance mucosal resilience focusing on tissue regeneration and healing and immune response.

**Methodology**

Atlantic salmon (*Salmo salar*) smolts (body weight 99,9 ± 13,8 g) were fed functional diets supplemented with one of the algae derived ingredients: *Ascophyllum nodosum* (whole biomass) (ASCOP), *Chaetoceros muelleri* (broken cells (CHAETO-BC), and water extract (CHAETO-WE)), *Skeletonema costatum* (broken cells) (SKE-BC), *Phaeodactylum tricornutum* (water extract) (PHAEO-WE), and a blend of CHAETO-BC and PHAEO-WE, in a total of six experimental diets and a control with no functional inclusion. Fish were fed for 14 days after which a portion of the anterior intestinal mucosa, skin mucosa (bellow dorsal fin) and gill mucosa (second arch's filaments) was excised and preserved in RNALater for gene expression analysis. A comprehensive molecular marker panel was set and evaluated, including genes related with general immune response (e.g. *CATH*, *MUC2*, *TLR22*, *CD82*), antioxidant response (e.g. *SOD*, *CAT*, *GPx*), epithelium homeostasis (e.g. *iFABP*, *OCL*, *TJP1*), and with the several stages of wound healing (re-epithelization and epidermal repair, inflammation, immune cell recruitment, cell proliferation and tissue remodeling). The expression of the panel of genes was assessed by RT-qPCR for the three mucosal systems, and relative expression was calculated with the Pfaffl's method. Univariate and multivariate analysis was performed, and diets were ranked based on the expression of the genes in each process, following a predefined set of rules for categorization of fish mucosal performance.

**Results**

By the end of the functional feeding trial, the expression of several genes in the GALT (intestinal mucosa) was modulated, but not significantly. However, diets with ASCOP and PHAEO-WE promoted a differentiated response in gut, mainly due to a stronger expression of *HSP70* and *TJP1* genes. In GIALT (gills), differences were more evident, and functional feeds promoted a shift in the gills mucosa when compared with CTRL feed. This shift was mainly due to an upregulation of *TJP1*, *OCL*, *TNFa*, *AGT*, *SOCS3* and *VEGFA* genes. On the other hand, SKE-BC diet resulted in downregulation of *IL1B*, *FNA1A* and *BNC2*, indicating a possible reduction in performance. In SALT (skin), the tissue remodeling related markers were modulated by the feeds, in particular the *PDGRFA* that was upregulated in diets with PHAEO-WE and ASCOP, and downregulated with SKE-BC. Here PHAEO-WE supplemented feed promoted a differential response mainly due to an upregulation of *FNA1A*, *PDGRF1*, *AGT* and *BNC2*, all related with epithelium regeneration and healing.

*(Continued on next page)*

**Conclusion**

The analysis and further ranking of the feeds indicated the PHAEO-WE supplemented feed as the best mucosal health promoter with higher performance score in GALT and GIALT, and as a second best in SALT. In skin, the diet supplemented with ASCOP promoted the greatest impact with a healthier response mainly in immune but also regenerative and healing processes. This study brought light to the potential of the algae-derived ingredients as mucosal health promoters in Atlantic salmon, with a special focus in the tissue regeneration and healing processes. However, the effects of these ingredients should be further addressed to fully evaluate if the positive outcomes here unravelled will reflect in higher performance and resilience after an insult.

**Acknowledgements**

This work is supported by the Horizon Europe project LOCALITY, funded by the European Union under Grant Agreement ID 101112884. Bernardo Carvalho is the recipient of a Ph.D. Scholarship supported by FCT (ref. 2022.12615.BDANA).

# LIPIDOMIC PROFILING OF GILTHEAD SEA BREAM (*Sparus Aurata*): IMPACT OF DIET COMPOSITION ASSESED BY UHPLC-IMS-HRMS

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**Introduction**

Lipids are fundamental to biological systems, serving as essential components of cellular membranes, signaling molecules and energy reservoirs. The biological functions of lipids are determined by their structural diversity. Diet plays a pivotal role in shaping the lipidomic profile, resulting in either harmful or beneficial health effects (1). To better understand the relationship between diet and health, there is a growing need for biomarkers that accurately reflect the consumption of health-relevant foods. Untargeted metabolomics can provide a more comprehensive image of dietary intake by profiling lipids in biological samples. High-resolution mass spectrometry (HRMS), with its high sensitivity and selectivity, is ideally suited for such analyses.

The aim of this work was to study the lipidomic profile of *Sparus Aurata*, one of the most consumed fish in the Mediterranean Sea, in response to diets differing in their main ingredients: fish-based, plant-based, by-product-based and alternative sources such as insect-derived components.

**Materials and methods**

Lipid extracts were obtained from freeze-dried fatty tissue of the fish fillet using the Folch method (chloroform, methanol and water), followed by reconstitution in isopropanol. Extracts were analyzed by ultra-high-pressure liquid chromatography coupled to ion mobility (quadrupole)time-of-flight mass spectrometry (UHPLC-IMS-QTOF MS), in both ionisation modes (ESI+/ESI-) using data independent acquisition (DIA) mode.

**Results**

Results show lipidomic differences among diets. Fish fed the control (CTRL) diet, based primarily on fish oil, showed higher levels of long-chain phosphatidylcholines (C18, C20). In contrast, those fed the alternative (ALT) insect-based diet exhibited a shift toward shorter-chain phosphoglycerides and diacylglycerides (C12, C16). This increase in short-chain lipids in the ALT diet aligns with the fatty acid composition of insect larvae, particularly *Hermetia illucens*, which is rich in lauric acid (C12:0) content (2, 3). In contrast, diets based on plant or animal sources resulted in higher levels of longer-chain fatty acids such as palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids (4).

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# UNTARGETED VOLATOLOMICS OF GILTHEAD SEA BREAM (*Sparus Aurata*) FOR DIETARY BIOMARKER DISCOVERY

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## Introduction

Nutritional quality is a key concern for the food industry, encompassing producers, regulators, and consumers alike. Volatile organic compounds (VOCs) are essential contributors to food aroma, freshness and perceived quality. Their profiles may be influenced by multiple factors, such as geographical origin, storage conditions, and dietary composition, among others (1, 2), making them useful indicators for food differentiation and traceability.

This study aimed to investigate the volatolomic signatures of *Sparus Aurata*, one of the most widely consumed fish species in the Mediterranean region, associated with three distinct diets containing novel, sustainable ingredients: plant-based, by-product-based and alternative sources such as insect-derived components. Understanding how diet influences the volatile composition of *Sparus Aurata* may contribute to the development of feeding strategies that preserve or enhance product quality, while also supporting the use of sustainable ingredients in aquaculture.

## Materials and methods

Two analytical extraction methods were applied for this purpose. First, a low-temperature extraction using head-space solid-phase microextraction (HS-SPME) at 60 ºC was performed to analyse the volatile compounds present in raw fish. Additionally, a high-temperature extraction via purge and trap (P&T) was carried out, simulating cooking by including a preheating step at 120 ºC followed by thermal desorption, to mimic the aroma profile perceived by the consumer.

## Results

The low-temperature analysis revealed clear differences in the volatile profiles among dietary groups. Notably, lauric acid was significantly elevated in fish fed the alternative diet. This biomarker is particularly relevant, as lauric acid is a C12 fatty acid commonly found in insects, the main ingredient of the alternative formulation, and has been previously reported in the volatolome of other fish species, such as salmon (3). The high-temperature analysis has been completed, and statistical evaluations are currently in progress. These results will provide further insight into how diet influences consumer-perceived quality through the fish's aroma profile.

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# COMPREHENSIVE SCREENING OF THE CONTAMINANTS IN FEEDS AND GILTHEAD SEA BREAM (*Sparus Aurata*) FILLETS BY GC AND LC COUPLED TO HRMS TECHNIQUE

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**Introduction**

Aquaculture plays a crucial role in addressing the increasing global demand for fish, requiring the development of sustainable feed formulations. Traditional diets are no longer sustainable and new nutritional strategies need to be studied (1, 2). However, incorporating alternative ingredients in feeds may introduce pollutants not commonly linked to fish farming.

This study examines the occurrence of potential pollutants across the marine food production chain—from raw ingredients to commercial feeds, and final fish fillets—using a feeding trial with gilthead sea bream.

**Materials and methods**

Eighteen raw ingredients, three different diets (each with two pellet sizes), and 48 fillet samples were analysed using advanced analytical platforms: liquid chromatography (LC) and gas chromatography (GC), both coupled to high-resolution mass spectrometry (HRMS), including ion mobility-quadrupole time of flight (IMS-QTOF) and GC-Orbitrap MS.

**Results**

A screening of different families of contaminants (pesticides, pharmaceuticals, etc.) was carried out. The results revealed the presence of typical plant-associated ingredients, such as pesticides and PAHs, in ingredients and feeds. Residues of veterinary pharmaceuticals were also detected in animal by-products ingredients, along with emerging contaminants like UV filters, musks, and phthalates. Interestingly, these contaminants did not show correlation with diet composition, suggesting contamination may originate from processing or storage. Notably, diets containing alternative ingredients showed lower contaminant loads compared to previous studies, with minimal transfer of pollutants to fish fillets. These findings highlight that alternative feed strategies can contribute to safer sustainable aquaculture practices without compromising consumer health (3).

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# DEVELOPMENT OF A PRIMARY CELL CULTURE FROM TISSUE TENTACLES OF SNAKELOCKS ANEMONE (*Anemonia viridis*) UNDER REGENERATION

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**Introduction**

The snakelocks anemone (*Anemonia viridis*) is a highly valued species in Spain due to its popularity as a seafood delicacy. Nevertheless, global warming, high exploitation and recent rapid growth of the invasive algae (*Rugulopteryx okamurae*) has left their populations in natural environments in critical condition. In fact, in October 2023, fishing for sea anemone was legally banned in south of Spain as a measure to protect the natural populations of this species. The importance of cell culture as a research tool is well recognized to study the cellular physiology of the organism under the effect of biological or environmental factors. The object of this study is to elucidate optimal conditions for the establishment of a primary cell culture of snakelocks anemone tissue. This will allow to develop an *in vitro* model to evaluate the effect of a physiological condition such clone generation on cell metabolism to be extrapolated to more complex models.

**Materials and Methods**

*Anemonia viridis* specimens were maintained under a sea water recirculation system with controlled temperature (20 ºC) and natural photoperiod (12:12). Two experimental conditions were established: (1) control and (2) induced regenerating by longitudinal section. After one week, one specimen of each condition was collected and four tentacles were dissected from each one. Following Ventura et al. (2018), two small pieces from each tentacle (2 $mm^2$) were cut, one of them seeded directly and the second one treated with collagenase IV (0.02%) before seeding, on modified Grace Insect Medium with 29.3 g/L NaCl (GMIM) with 5% foetal bovine serum, 1% penicillin/streptomycin, 1% amphotericin B, 0.1% Tylosin and 73% sterile artificial seawater. Four types of cultures were tested: control-collagenase, control+collagenase, regeneration-collagenase, regeneration+collagenase (four replicates from each one). Culture conditions were: atmospheric $pCO_2$, Tª 20.0 ± 1 ºC and darkness. Cells were monitored until day 34 under inverted optic microscopy (Leica DMi1). Images were analysed to measure cellular homogeneity (Fiji v.2.17.0.). Mixed-design ANOVA ($p$<0.05) was performed using SPSS v.25.

**Results and discussion**

One of the most important factors on primary cell culture establishment is the growth capacity of tissue. Also, tissue treatment with collagenase IV is a key factor in the digestion of the extracellular matrix when isolating cell culture. In the present study, results revealed the presence of three types of cells in *A. viridis* tentacle tissue culture (Figure 1): epithelial cells (more abundant), cnidocytes (defence cells) y zooxanthellae (endosymbiotic cells from *Symbiodinium* genus).

The homogeneity index (percentage of epithelial cells over the total cells), evaluated from 24 hours to 34 days, displayed that at day 8 all of the groups reached values of homogeneity higher to 95 % (Figures 2). The relationship between physiological status of tentacle tissue (control or induced regeneration) and collagenase treatment, affected the evolution of the culture of epithelial cells at different time points. Control condition treated with collagenase, displayed and early cell homogenization (24 hours) compared to regeneration groups treated with collagenase, which, by other hand, reached higher values of homogeneity on later stages compared to control. This could be due to physiological condition of cells, on one hand, control under basal metabolism favoured a quick development of epithelial cells while regeneration has to deal with stressed physiological status (induced section on tissue) and enzymatic digestion, resulting in a slow beginning but steady increase in culture quality and the end of

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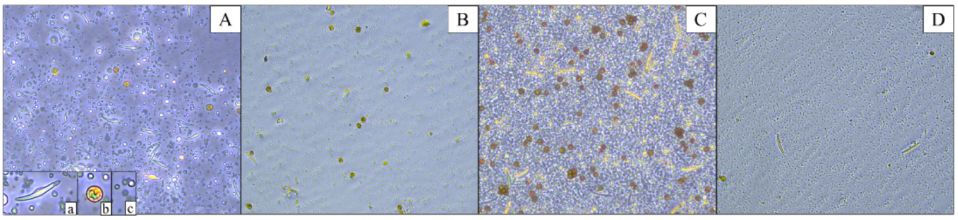


**Fig. 1** Culture cells from tentacles of *A. viridis* treated with collagenase IV. Control group (A: 24 h, B: 34 days) and regenerating induced group (C: 24 h, D: 34 days). Three types of cells can be identified (A): a: zooxanthellae, b: epithelial cells, c: cnidocytes. Observations done on inverted optic microscope with 20x.

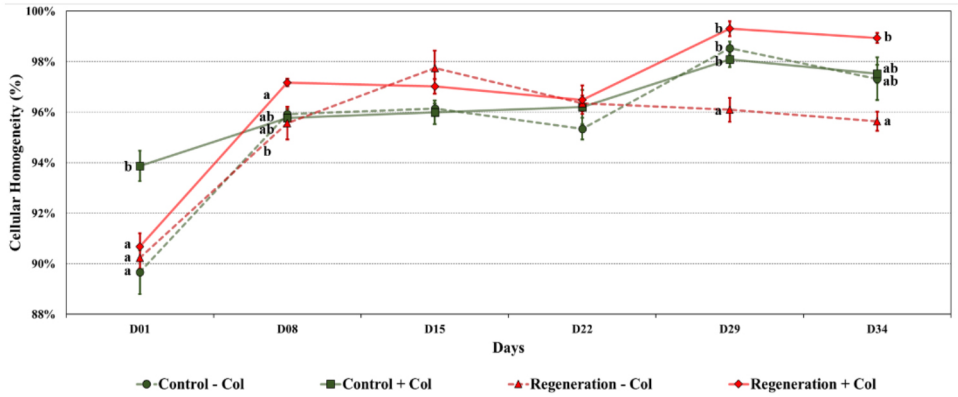


**Fig. 2** Homogeneity index of epithelial cells ((total nº of epithelial cells / total nº of cells) x 100) in primary cell cultures from *A. viridis* tentacles. Time lapse from 24 h to 34 days. control (not induced groups), regeneration (induced groups), - col (no collagenase treatment), + col (collagenase treatment). a, b, c: significant differences between experimental treatments ($p$<0.05).

the experimental time (34 days). Similarly, control and regenerating groups without collagenase treatment increased values of homogeneity over time, but in both groups, values were lower in early stages compared to collagenase treated groups. being this difference significant at the end of the experimental period in regeneration groups, which could explain an affected capacity of proliferation in cells not treated with this enzyme under an adverse condition. This study concludes that the metabolic status of source tissue can be determinant on the effect of collagenase, promoting an early or later development of the homogeneity and quality of the culture.

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# ASSESSING IMPACTS OF 14° C VERSUS 17° C REARING TEMPERATURE ON COHO SALMON *Oncorhynchus kisutch* GROWTH PERFORMANCE, HEALTH, AND WELFARE IN FRESHWATER RECIRCULATION AQUACULTURE SYSTEMS

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## Introduction

The growing land-based salmon industry, utilizing recirculation aquaculture system (RAS) technologies, has largely focused on producing Atlantic salmon *Salmo salar*. In the United States, there are presently only two such facilities raising alternative salmonid species – steelhead trout *Oncorhynchus mykiss* and coho salmon *Oncorhynchus kisutch*. While steelhead trout production benefits from decades of selective breeding efforts, an abundance of performance data, and year-round egg availability, coho salmon is a relatively unstudied species. Nonetheless, there is significant interest from RAS stakeholders in coho salmon as an alternative salmonid species with considerable market potential. Early efforts in raising coho salmon in RAS in the U.S. demonstrated disappointing growth performance well below targeted levels due to suboptimal rearing environments; however, more recent advances in rearing protocols have shown that excellent growth performance can be achievable in RAS. Nonetheless, optimized culture conditions for coho salmon production in RAS still need to be defined.

## Materials and methods

All-female diploid coho salmon eyed eggs were obtained from a U.S. commercial supplier and hatched in Heath trays within a RAS incubation system maintained at 8-10 °C. After the sac fry has absorbed the majority of their yolk sacs, they were transferred to six 0.5 $m^3$ tanks in a flow-through system (approximately 13.5 °C). Following normal industry protocols, all salmon were raised under a natural, rising photoperiod (Campbell River, BC, Canada latitude) from hatch until 30 weeks post-first feeding, in order to instigate smoltification beginning at approximately 50 g. At 30 weeks post-first feeding, the entire population was bottom-culled to eliminate fish that failed to undergo smoltification. Fish remained on natural photoperiod in the flow-through system until a mean weight of 600 g was achieved, after which they were transferred to six replicated RAS (9.5 $m^3$ total volume; 5.3 $m^3$ tanks) maintained at either 14 °C or 17 °C, with n=3 replication for high vs. low-temperature treatments. All fish were fed standard diets from a commercial supplier for the duration of the study; feed amounts provided to each study tank over the course of the experiment were based on standard industry feeding charts, but were adjusted based on feeding activity such that fish were fed to satiation. All RAS were operated at an exchange rate that facilitated 17 °C water temperature, with chillers being employed in the lower temperature group to achieve 14 °C in those specific RAS. Throughout the study period, fish performance was / will be assessed bimonthly, quantifying length, weight, dorsal and caudal fin condition, and cataract scores from a representative sample of fish per tank. The study will be completed once the best-performing treatment group attains a mean weight of 3 kg (i.e., the targeted industry harvest size). Aside from the performance outcomes listed above, maturity will be assessed via gonadosomatic index calculation from a representative sample of fish from each tank, and viscerosomatic indices will be quantified to assess coelomic adipose deposition. Mean condition factor and population coefficient of variation (weight) will be calculated at each sampling point, and specific growth rate, thermal growth coefficient, and feed conversion will also be assessed. Background water chemistry for relevant RAS parameters (alkalinity, BOD, hardness, pH, $NO_2$-N, $NO_3$-N, total phosphorus, TSS, true color, and turbidity) are being regularly assessed throughout the study period.

## Results

At the time of abstract submission, the most recent sampling event was carried out at around 1 kg mean weight, and final sampling at 3 kg is projected to be completed in June-July 2025. Presently, no clear differences in the performance, health, and welfare metrics listed above have been noted between the two treatment groups; however, all final results will be presented at Aquaculture Europe 2025.

# ENSURING OPTIMAL WATER QUALITY IN RAS ENGINEERING USING HIGH-FIDELITY MODELING

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Water quality plays a fundamental role in fish health, growth, and overall system performance in Recirculating Aquaculture Systems (RAS). Among the various water quality parameters, dissolved oxygen (DO) is one of the most critical. Fish require a stable and adequate supply of oxygen throughout the tank volume, yet maintaining this can be challenging in high-density aquaculture systems. Uneven oxygen distribution, such as too high levels around inlets, oxygen-depleted zones near the tank bottom, and the presence of biosolids can create microenvironments that stress fish, reduce feed conversion efficiency, and increase disease susceptibility.

This study highlights the role of DO distribution and water quality management in RAS. The model-visualizes and analyzes how oxygen is distributed in a commercial RAS tank under typical operating conditions, also accounting for the impact of the fish itself. The model accounts for stocking density, fish oxygen consumption and fish distribution, and shows how variations in water flow influences the oxygen availability in different parts of the tank. Figure 1 shows the model prediction of DO distribution across the central vertical plane for a salmon stocking density of 64 kg/m3. By simulating various conditions such as biomass densities, oxygenated water inlet placements, and waste accumulation patterns, it is possible to identify practical strategies to reduce oxygen gradients and promote more uniform conditions throughout the tank. These include optimizing the placement of oxygen delivery systems, improving tank flow design, and regularly removing biosolids to prevent stagnation.

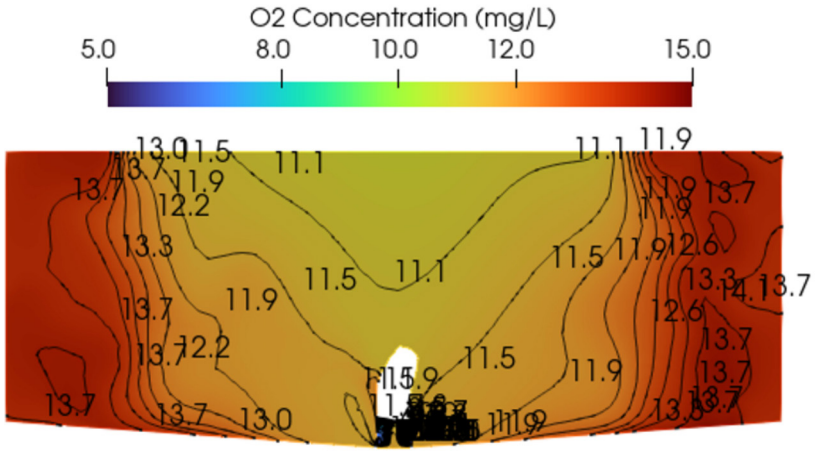


**Figure 1: CFD-prediction of oxygen distribution in a commercial RAS tank across the central vertical plane.**

# EVALUATING NUTRIENT TRANSFER AND PRODUCTIVITY IN A CLOSED-LOOP AQUAPONICS SYSTEM USING CATFISH AND ROMAINE LETTUCE

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## Introduction

Aquaponics integrates fish farming with soilless plant cultivation (hydroponics) in order to establish a symbiotic system where fish waste provides nutrients for plants, with bacteria facilitating the conversion of ammonia to nitrates. In contrast, hydroponics involves plant growth using nutrient-enriched water. This study aims to compare the growth rates and production of lettuce and fish within an aquaponics system.

## Methods

Catfish (*Ictalurus punctatus*) were raised under controlled conditions, receiving a daily feed of 3% of their body weight, while Romaine lettuce (*Lactuca sativa var. longifolia*) seedlings were transplanted into a system utilizing reverse osmosis-treated water. Daily monitoring of total dissolved solids (TDS) and electrical conductivity (EC) ensured nutrient optimization. Water samples were tested for ammonia, nitrite, nitrate, pH, and dissolved oxygen using API strips.

## Results

Preliminary results from the catfish showed an average weight of 56.74 ± 15.71gm (Figure 1). Lettuce growth displayed an average weight of 17.76g ± 9.85gm. These findings suggest that the aquaponic system facilitates effective nutrient transfer from the fish tank, promoting lettuce growth. Future research will compare these results to soil-grown lettuce and pond-grown fish to evaluate which method produces higher growth and yields, providing insights into sustainable agricultural practices for both leafy greens and fish production.

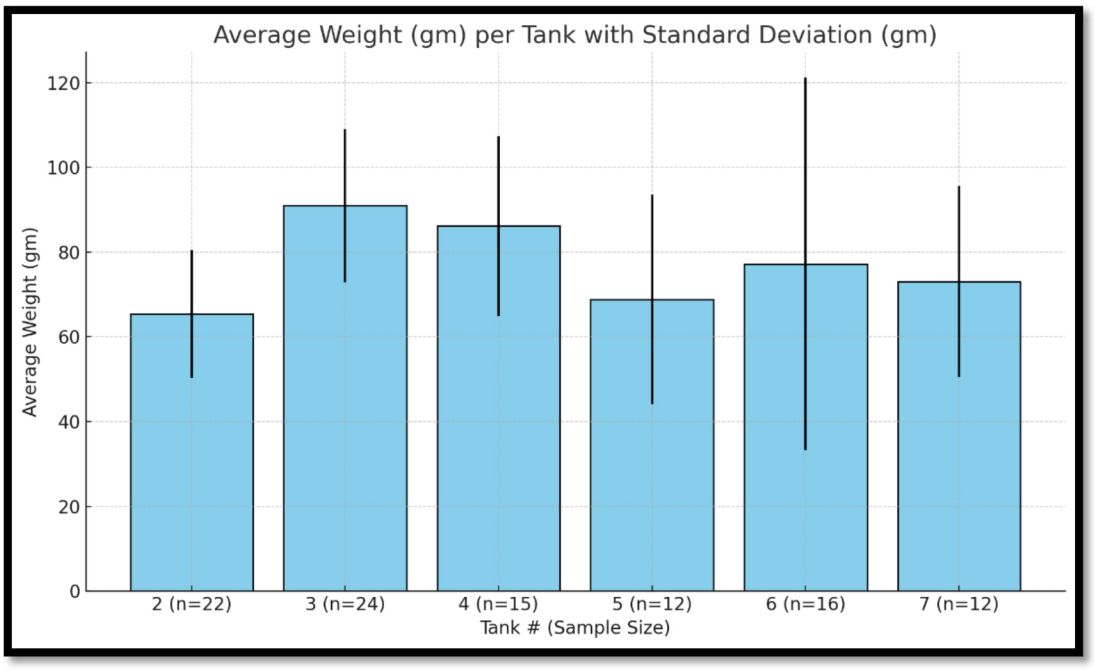


**Figure 1.** Average weight (gm) of fish per tank with standard deviation (gm). Sample size for each catfish tank is indicated in parentheses next to the tank number.

# OPTIMIZING CARBON STORAGE IN AQUACULTURE SYSTEMS: EVALUATING FISH SPECIES SELECTION AND FARMING PRACTICES

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## Introduction

Aquaculture ponds, often neglected in national carbon budgets, present significant potential for carbon storage. This study investigates the carbon absorption rates of pond sediments in relation to fish species selection, pond age, and fish farming practices.

## Methods

A standardized soil sampling protocol was employed, collecting sediment samples of 200-300g from a depth of 20 cm using a hand core sampler, based on the methodology from Gilbert et al., 2021. To ensure consistency, all samples were collected by the same individual and analyzed at the Oklahoma State University Soil Testing Lab. Soils from channel catfish, koi, and bluegill ponds were sampled, with four catfish ponds, two koi ponds, and two bluegill ponds included in the study. A questionnaire gathered data on physical, chemical, and fish farming management factors.

## Results

The results showed that carbon absorption rates varied significantly between species, with bluegill ponds having the highest organic matter and total carbon content, followed by catfish and koi ponds. Carbon absorption was also analyzed in relation to pond age and management practices such as stocking rate, feed, and total production, with bluegill ponds again demonstrating the highest absorption rates. This study underscores the potential for improving carbon storage in aquaculture ponds, emphasizing the importance of fish species, pond age, and management practices in carbon sequestration strategies. These findings offer valuable insights into the role of aquaculture systems in mitigating climate change impacts.

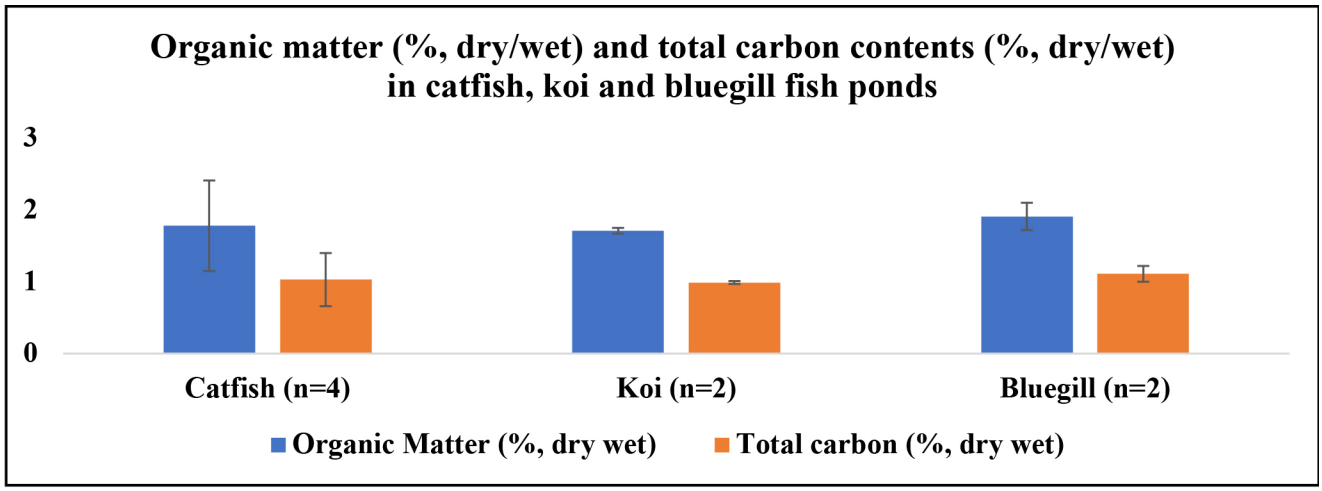


**Figure 1: Organic matter (%, dry wet) and total carbon contents (%, dry wet) in channel catfish, koi and bluegill fish ponds (source: current research)**

# IMPACT OF CLIMATE-DRIVEN ENVIRONMENTAL CHANGES ON AQUATIC ECOSYSTEMS AND DISEASE OUTBREAKS

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## Introduction

Climate change is significantly impacting aquatic ecosystems, altering species distribution and abundance, and influencing disease dynamics. Rising temperatures and environmental factors such as population density and water quality are contributing to the spread of antimicrobial resistance (AMR), with both terrestrial activities and human practices, like agriculture and urban waste, exacerbating the issue. Addressing AMR in aquaculture, a sector highly influenced by climate variability, requires a comprehensive approach that incorporates sustainable practices and proactive disease management.

## Material and Methods

We conducted a double meta-analysis of 460 scientific publications to assess the impacts of global warming and antimicrobial resistance (AMR) on aquaculture. Our analysis included 11,274 bacterial strains linked to aquaculture across 40 countries. From this, we calculated a Multi-Antibiotic Resistance (MAR) index, revealing that AMR levels are particularly high in low- and middle-income countries.

## Results

Our results show that aquaculture MAR indices are positively correlated with clinical AMR levels, environmental temperatures, and national climate vulnerability. Additionally, we found that disease-related mortalities in aquatic animals tend to increase at higher temperatures.

## Conclusion

Countries most vulnerable to climate change are likely to face the highest risks from antimicrobial resistance (AMR), with potential impacts extending beyond aquaculture to human health. This underscores the urgent need for sustainable strategies that reduce antibiotic use and enhance the resilience of aquaculture systems.

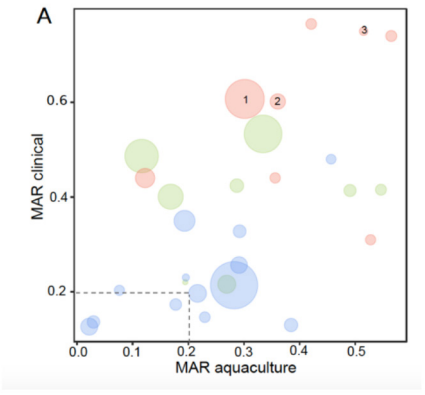


Figure 1. Correlations between MAR calculated from aquaculture-related bacteria and human clinical bacteria, temperature, and countries' climate vulnerability. Pearson correlations (two-sided test) a MAR from human clinical bacteria (n = 29, P-value < 0.001) and c temperature (n =40, P-value 0.10). Bubbles sizes are proportional to national aquaculture production standardised by the total country human population.

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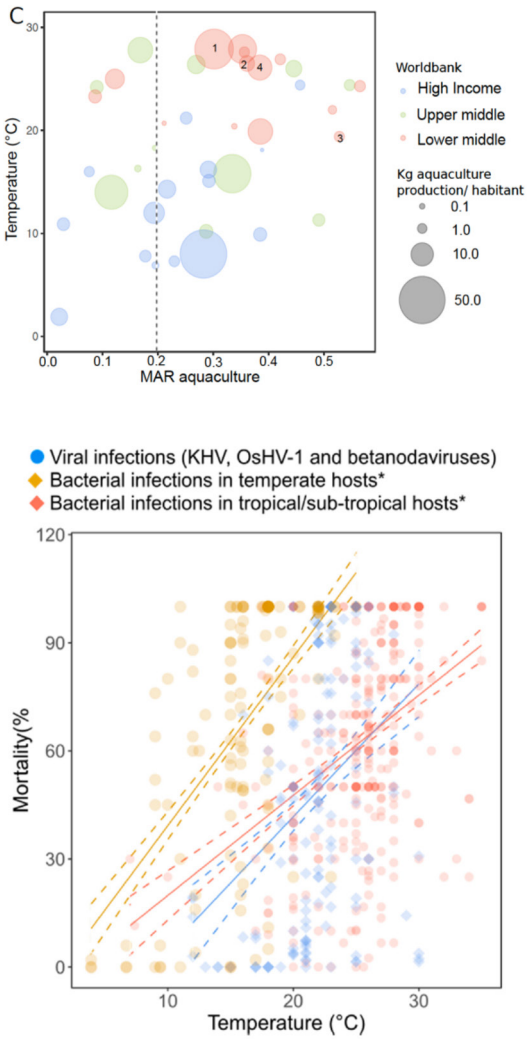


Figure 2. Predicted changes in mortality (%) of reared aquatic animals infected by bacteria (yellow and red and viral (blue, ostreid herpesvirus, koi herpesvirus and betanodaviruses) diseases in response to temperature. Solid lines represent the predicted mortalities according to selected linear and linear mixed models with dotted lines representing the 95% confidence interval for each of the models.

**Refererece**

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Combe et al. Impact of GlobalWarming on the Severity of Viral Diseases: A Potentially Alarming Threat to Sustainable AquacultureWorldwide. Microorganisms 2023, 11, 1049.

# THE BLUEBOOST PROJECT: THE SARDINIAN CASE STUDY

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The BLUEBOOST project promotes the development and validation of innovative Integrated Multi-Trophic Aquaculture (IMTA) systems as a solution to improve sustainability in the aquaculture sector. By co-cultivating species from different trophic levels such as fish, bivalves, macroalgae, and other invertebrates. IMTA systems enhance nutrient recycling, reduce environmental impacts and contribute to a more circular and climate-resilient Blue Economy.

In Italy, the Sardinian case study is coordinated by the International Marine Centre (IMC) and is located in the Gulf of Oristano, a mesotrophic coastal environment in the central-western Mediterranean. The pilot system integrates an existing commercial fish farming facility rearing species such as European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and meagre (*Argyrosomus regius*) with the cultivation of the European flat oyster (*Ostrea edulis*), a native species of high ecological and commercial value.

As part of this IMTA system, oyster farming gears, including lantern nets and Australian baskets, have been suspended at 3 m depth in close proximity (~30 m) to the fish cages. The goal is to evaluate the growth performance of *O. edulis* and to study potential trophic interactions between the fish species and oysters. Water quality and stable isotope analyses have been conducted to assess nutrient flows and the ecological functioning of the integrated system. Furthermore, local hydrodynamic conditions were evaluated to elucidate water circulation patterns and associated particle dispersion dynamics, thereby enhancing the interpretation of isotopic signatures and better assess trophic connectivity within the IMTA setup.

To complement these investigations, microbiological studies were also carried out on surface sediments, with seasonal sampling. Using next-generation sequencing (NGS), the diversity and structure of benthic bacterial communities were analyzed across multiple stations influenced to different extents by the IMTA system.

In addition to field activities, a crucial component of the Sardinian IMTA system is the production of *O. edulis* spat at IMC's experimental hatchery. The hatchery is equipped with dedicated units for phytoplankton cultivation, broodstock conditioning, larval rearing, settlement and nursery. IMC successfully completed the full hatchery cycle starting from locally harvested broodstock, gamete induction and fertilization, larval metamorphosis and juvenile growth, until producing over 5,000 spat that were transferred to sea for on-growing once they reached an average shell length of 18.7 mm.

This production process has also led to the drafting of a technical protocol for *O. edulis* seed production, with potential application in small-scale commercial aquaculture and oyster restoration initiatives. The protocol covers key aspects such as biosecurity, water quality control, feeding regimes, and grading procedures, providing a practical tool for hatcheries aiming to produce robust and healthy oyster seed.

The results of this pilot, together with those of five other case studies across Europe and South America, will feed into broader evaluations of IMTA's environmental and economic performance using Life Cycle Assessment (LCA) and other multidisciplinary tools. Through this integrated approach, BLUEBOOST aims to facilitate the adoption of IMTA systems at commercial scale and inform future policy and investment in sustainable aquaculture practices aligned with European climate and biodiversity strategies.

# TOWARDS A SUSTAINABLE AND COMPETITIVE EUROPEAN AQUACULTURE SECTOR: BREEDING AND GENETICS RESEARCH PRIORITIES FROM FABRE TP

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## Introduction

Aquaculture plays a central role in the European Union's ambitions to build sustainable food systems, secure protein sources, and support economic resilience across its regions. Yet the sector faces major challenges—ranging from climate change and environmental degradation to increased global competition and evolving consumer expectations. Breeding and genetics are key tools to address these challenges while improving productivity, animal welfare, and ecological efficiency. The Farm Animal Breeding and Reproduction Technology Platform (FABRE TP) has developed a strategic framework to guide research and innovation efforts in this field, based on a shared vision for competitive, resilient and responsible aquaculture.

## Methodology

The FABRE TP Aquaculture Research Priorities were developed through a participatory process combining members' expertise, analysis of recent EU-funded projects (such as AquaFAANG, FISHBOOST, AquaExcel), and targeted stakeholder consultation like EATiP. A dedicated survey in mid-2024 gathered insights from 23 experts across 11 countries, identifying focus traits, key research gaps, and strategic directions. These inputs were synthesised into a structured roadmap aligned with the four pillars from the *Strategic Guidelines for Sustainable and Competitive EU Aquaculture (2021–2030)*, adapted to the context of breeding and genetics.

The priorities were officially presented at the SCAR FISH meeting in March 2025 and at FABRE TP's Annual Meeting in Brussels, where they were positively received by representatives from DG RTD. The entire process was guided by the principle that collaboration between private breeding organisations, academic research supported by public institutions is essential to delivering impactful, long-term change.

## Strategic Pillars and Priorities

1. **Building resilience and competitiveness**
   Aquaculture systems must adapt to environmental variability—such as temperature fluctuations, water quality shifts, and disease outbreaks—while remaining economically competitive. Priorities under this pillar include genetic adaptation to new rearing environments (e.g. RAS, IMTA), improving multi-trait disease resistance, supporting the domestication of emerging species including algae and low-trophic species, and safeguarding genetic diversity. Developing tools to monitor and limit introgression between farmed and wild populations and expanding the use of reproductive and genomic technologies are also crucial.
2. **Engaging in the green transition**
   Genetics can play a transformative role in reducing aquaculture's environmental footprint. This includes breeding for feed efficiency, resilience to climate change, and compatibility with circular systems. Innovations in feed ingredients—such as reduced fishmeal and fish oil—require genetic lines that can adapt nutritionally. Genetic co-selection strategies, sterility techniques, and genomic tools to improve traits like stress tolerance and mortality reduction are integral to supporting climate-smart aquaculture.
3. **Promoting social acceptance and consumer trust**
   Transparency, ethical considerations, and communication are central to ensuring social license for aquaculture. Responsible breeding must be paired with public understanding of its benefits for sustainability, animal welfare, and food security. Research is needed to identify and address the social, environmental, and economic dimensions that influence acceptance of breeding technologies—particularly for low-trophic and newly domesticated species. Clear policy frameworks and evidence-based regulation will help navigate innovation while maintaining trust.

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4. **Advancing knowledge and innovation**
   A more innovative, data-driven, and collaborative research ecosystem is needed to sustain genetic progress. Priorities include developing automation, sensors, and artificial intelligence to support phenotyping and trait monitoring; applying genomic and gene editing technologies within appropriate risk and ethical frameworks; and strengthening collaborations across public, private, and academic actors. Special attention is needed to expand capacity and infrastructure in EU Outermost Regions (ORs) and Overseas Countries and Territories (OCTs), where climate-adapted breeding programs can support local production and competitiveness.

**Conclusion**

The survival and adaptability of aquaculture species are cross-cutting challenges that touch all four pillars of this strategy. Addressing them requires long-term investment in breeding programmes and an integrated research and innovation agenda. FABRE TP calls for coordinated action at the EU level—supported by instruments such as Horizon Europe—to fund science and translate it into solutions that are socially accepted, environmentally sustainable, and economically competitive. Collaboration between the private sector, public institutions, and academia is essential to ensure that Europe reinforces its role as a global leader in breeding innovation and improves its competitiveness for sustainable aquaculture.

# FISHING FOR BRAINWAVES: HOW TO CATCH A CLEAN EEG SIGNAL WITHOUT GETTING TANGLED IN NOISE

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**Background**
The growing recognition of fish as sentient beings has led to increased attention to fish welfare in both aquaculture and wild capture contexts. A key area of concern is the period of stunning and killing, where ensuring unconsciousness prior to death is critical for securing humane treatment of animals. Humane killing is not only an ethical imperative but also a foundational principle in animal welfare legislation. In this context, the European Food Safety Authority (EFSA) highlights the use of neurological indicators obtained via electroencephalography (EEG) as the gold standard for assessing the effectiveness of stunning. Nevertheless, commercial practices often rely on operational indicators such as cessation of movement or loss of equilibrium. Emerging evidence suggests that these signs can be misleading; in many cases, fish may appear immobile while EEG recordings reveal ongoing brain activity indicative of consciousness (van de Vis et al., 2003). This discrepancy underscores the urgent need for reliable, objective tools to assess stunning efficacy.

**Material & Methods**
To address this need, we adapted EEG methodologies established in humans to develop a non-invasive technique suitable for use across several fish species. Our setup employs a silicon suction cup to attach plate electrodes securely to the head of the fish, ensuring stable contact without surgical implantation. This approach enables us to record cortical electrical activity with minimal interference to the animal (Brijs et al. 2021; Hjelmstedt et al. 2022). However, EEG recordings in fish are prone to signal contamination. External noise sources include electrical interference from surrounding equipment, variability in electrode material, and characteristics of connecting wires (e.g., shielding, length). Physiological noise, such as muscle contractions, cardiac activity, and respiratory movements, further complicates signal interpretation. These artifacts can mask or mimic genuine neural activity, increasing the risk of misinterpretation.

**Results**
Through controlled laboratory trials, we evaluated and reduced noise from external sources by optimizing equipment setup, electrode placement, and shielding. This improved baseline signal quality and reduced variability. Physiological artifacts presented more complex challenges. Our data demonstrate that muscle activity can dominate the EEG trace (Figure. 1). For example, ventilatory muscle activity can introduce low-frequency artifacts that may bias interpretations, especially in stunned but not fully unconscious fish. To improve reliability, we incorporated event-related potentials (ERPs), specifically visual evoked responses (VERs), into our assessments. Unlike spontaneous EEG, ERPs are time-locked to external stimuli, allowing for averaging over time to reduce background noise. Our findings show that VERs provide a robust method for detecting unconsciousness, as they reflect whether external stimuli are still being processed by the brain. This technique significantly enhances the interpretability of EEG data under noisy conditions.

**Conclusions**
Our work highlights the risk of misinterpretation when EEG recordings are evaluated without accounting for noise and artifacts. This is particularly critical in the context of animal welfare assessments, where the consequences of false-negative or false-positive conclusions about consciousness are ethically significant. As EFSA recommends, combining spontaneous EEG analysis with ERP-based assessments significantly enhances reliability and should become standard practice in evaluating fish stunning methods. To move this field forward and establish EEG-based methods as credible tools for welfare assessment, we must also raise the bar for methodological transparency and rigor. By ensuring this level of detail and standardization, we can promote reproducibility, facilitate cross-study comparisons, and build a stronger scientific foundation for evidence-based fish welfare policy. Ultimately, EEG-based assessments, when combined with ERP methodologies and conducted with high methodological precision, offer a powerful approach to objectively and ethically evaluate stunning efficiency in fish. These tools not only serve scientific progress but also address growing societal and regulatory demands for transparency and compassion in animal handling.

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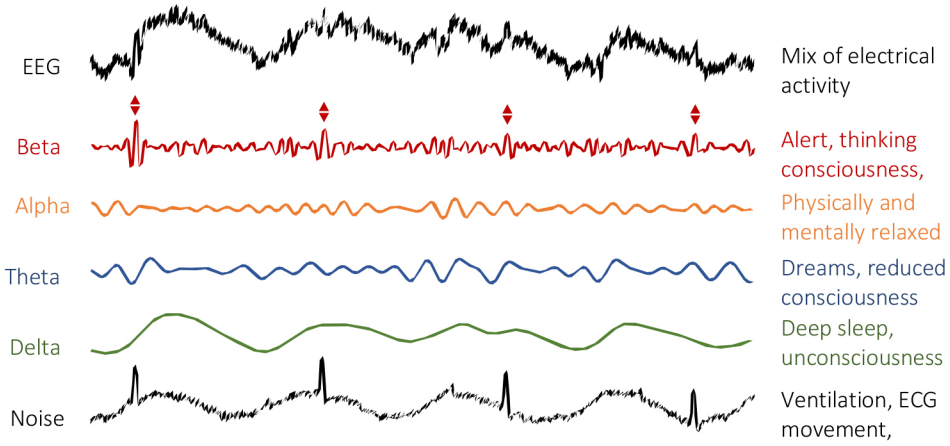


**Figure 1.** Brain waves are rhythmic patterns of electrical activity in the brain, shown here in EEG recordings from Rainbow trout (*Oncorhynchus mykiss*). These waves differ in frequency and are commonly linked to various states of consciousness and brain function. When EEG is recorded alongside the raw bioelectrical potential from the surrounding water, it becomes evident that physiological noise introduces artifacts across several frequency bands. Notably, electrical activity from ventilatory muscles strongly interferes with the lower-frequency delta range, often dominating the signal in that band.

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# ASC SUSTAINABLE AQUACULTURE: CREATING SUPPORT MATERIALS FOR THE WELFARE PRINICIPLE IN THE NEW FARM STANDARD

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Aquaculture plays a critical role in the global seafood trade, contributing substantial economic value and food safety. Over the years, the industry has experienced substantial growth and is projected to continue expanding in the future. Ensuring the long term sustainability and longevity of aquaculture operations depend on the widespread adoption of responsible and sustainable farming practices that prioritize the health and welfare of aquatic species throughout their lifecycle.

In this context, the Aquaculture Stewardship Council (ASC) provides a globally recognized certification and labelling program for responsibly farmed seafood. ASC's certification program empowers farmers by enabling them to differentiate their products, expand market access, and build consumer trust through verified responsible practices

To strengthen its approach, ASC has brought together its twelve species-specific standards into one robust Farm Standard to provide greater consistency. This consolidated Farm Standard is structured according to four principles – with the new, fourth principle developed to put fish welfare front and centre of the new approach.

The welfare principle will include criteria on health and welfare related to the daily operations at the farm, handling and slaughter requirements. The welfare requirements have monitoring stipulations, however given the diversity of farmed species, production systems and current gaps in scientific data, no new set parameters for stocking density etc have been included in the standard. Instead, it introduces a novel, flexible traffic light system that is tailored to accommodate the species being farmed, their life stage and the unique farm context. Farms are required to define Acceptable, Warning Levels and Unacceptable Thresholds for the traffic light system for each requested operational welfare indicator, promoting context specific management and continuous improvement.

The presentation will focus on discussing the rationale for adding the traffic light system for specified welfare requirements to the standard as well as the tools created to support the implementation on farm.

# DEVELOPMENT OF NEW AQUAFEED FORMULATIONS WITH HIGHER NUTRITIONAL EFFICACY AND SUSTAINABILITY (PCM - 00044)

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**Aim of research**

Fish aquaculture is highly dependent on the use of compound feeds, with national consumption in Spain reaching 181,000 tonnes in 2023. Fishmeal remains the predominant protein source in aquafeeds and has almost doubled its cost over the past two decades due to increasing demand combined with decline in fishery catches. This has promoted the search for alternative protein sources. Insect protein is a sustainable alternative with nutritional composition (75% w/w protein in defatted insect meal) comparable to fishmeal. Insect farming is sustainable and presents high viability due to its high feed-to-protein conversion efficiency. To date, several insect species such as soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*) have been evaluated as fishmeal replacers in aquafeed formulation. Another concern for aquafeed industry is the supplementation with functional ingredients such as peptides, fatty acids, vitamins, or probiotics. In this regard, protein hydrolysates provide low-molecular weight peptides which improve both digestibility and palatability of aquafeeds, improving feed utilization and therefore growth rate. Besides this, peptides can display specific biological activities, such as antimicrobial, anti-inflammatory or antioxidant, among others. Supplementation with bioactives (i) restrains lipid oxidation of aquafeeds, (ii) helps mitigate the oxidative stress caused by the environmental factors or (iii) avoid the extensive use of antibiotics.

The project "Development of New Aquafeed Formulations with Higher Nutritional Efficacy and Sustainability (PCM_00044)", funded by the Ciencias Marinas programme, aims at the formulation of new aquaculture diets with inclusion of partially hydrolysed insect (*Tenebrio molitor*) protein. Three functional ingredients were included in the diet formulation: (i) Arachidonic acid, which is deficient in fish oil commercial preparations, (ii) sunflower seed hydrolysates displaying *in vitro* antioxidant and anti-inflammatory activities, (iii) allium-derived extract with antimicrobial activities against fish pathogens.

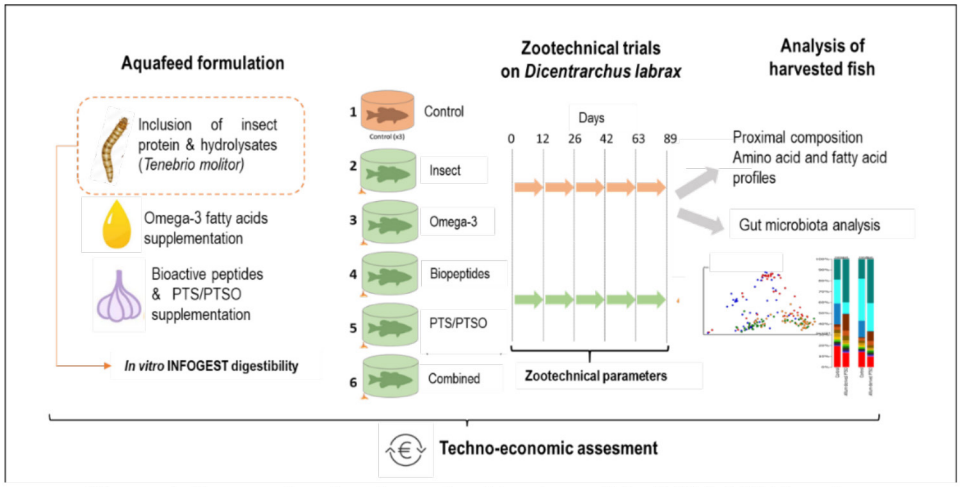


**Figure 1.** Layout showing the main objectives of the PCM_00044 project

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**Materials and Methods**

The project PCM_00044 engages a transdisciplinary consortium, led by the BIO-110 Research Group from the Department of Chemical Engineering at the University of Granada (UGR) with the collaboration of the BIO-160 Group from the Department of Microbiology (UGR) and the Aquaculture Technology Centre of Andalusia (CTAQUA). Two private partners are involved in the project, DMC Research Center Ltd. and TEBRIO Ltd. as suppliers of the allium-derived extracts and the defatted mealworm meal, respectively. As shown in the Figure 1, the project tasks are distributed into four main workpackages (WP), devoted to the formulation of aquafeeds and *in vitro* validation by the INFOGEST method adapted for European seabass (*Dicentrarchus labrax*). The lipid ingredient was formulated by enrichment of the standard fish oil with ARASCO® (DSM Nutritional Products, Switzerland) to meet the nutritional recommendations for larval and juvenile rearing. Sunflower (*Helianthus annuus*) seed hydrolysates (DH 10%) and a blend of extracts derived from *Allium spp.* (PTS/PTSO) were chosen as bioactives.

Six experimental diets were tested through a 12-week feeding trial in CTAQUA, involving 1,170 seabass juveniles (initial weight: 3.25 ± 0.33 g) reared in an 18-tank (100 L each) recirculating aquaculture system (RAS): (D1) a commercial control diet for pre-growing seabass, (D2) 25% w/w of the protein replaced by *T. molitor* protein partially hydrolysed (15% w/w of protein); (D3) diet supplemented with ARA; (D4) diet with sunflower seed peptides; (D5) diet containing allium-derived extract and (D6) a combined diet (insect protein, ARA and biopeptides). Growth performance was monitored throughout the trial, with immunological assessments conducted at termination. The analysis of the harvested fish after feeding trial had two goals: (i) nutritional evaluation of fish flesh and lipid content and (ii) analysis of intestinal microbiota DNA and mucus. Finally, the feasibility of the new aquafeed formulations was estimated by a techno-economic assessment.

**Results and discussion**

The experimental diets presented no significant differences against control diet in terms of survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), Fulton's condition factor (K) and hepatosomatic index (HSI). The survival rate exceeded 95% for all the diets, with a final body weight above 40 g at the end of the feeding trial. Harvested fish flesh and oil presented no significant differences among experimental diets. The fatty acid profiles show significant levels of omega-3 fatty acids (EPA, DHA, ALA) The muscle amino acid profile stands out for the content of essential amino acids (29–31% w/w).

**Conclusion**

This study supports the feasibility of replacing fish meal by partially hydrolysed insect meal, as well as supplementation with functional ingredients such as omega-3 fatty acids, plant biopeptides and allium-based extracts in European seabass feeds. Immune response and gut microbiota profiling analysis —currently in progress— are necessary to confirm the effect of the experimental diets on reared fish.

**Acknowledgement**

This research was funded by the project PCM_00044, granted by the Consejería de Universidad, Investigación e Innovación (Junta de Andalucía), Government of Spain and NextGenerationEU-European Union. DMC Research Center Ltd. and TEBRIO Ltd. are acknowledged for their scientific advice and the provision of defatted mealworm meal and allium-derived extracts, respectively.

# FINE-TUNING SPOROVACCINES TO IMPROVE ORAL IMMUNISATION IN THE AQUACULTURE SECTOR

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## Introduction

The aquaculture sector has experienced remarkable growth in recent decades. In 2022, global aquaculture production of aquatic species surpassed capture fisheries for the first time, highlighting the sector's vital role in meeting global food demand (FAO, 2022). However, bacterial diseases continue to pose a significant threat to sustainable aquaculture, resulting in substantial animal losses and the overuse of antibiotics (Santos & Ramos, 2018). As antibiotic resistance increases, developing effective alternatives is important for safeguarding human, animal, and environmental health. Vaccination is the most sustainable approach for disease prevention, consequently reducing the industry's dependence on antibiotics. Among the various immunisation strategies employed in aquaculture, oral vaccination stands out for its ease of administration, and suitability for mass delivery, particularly to larvae and juvenile fish (Mondal & Thomas, 2022).

This project researches the use of *Bacillus subtilis* spores as vehicles for delivering heterologous antigens to the fish gastrointestinal tract (Gonçalves et al., 2022). We aim to utilise this spore-based vaccination system for the prevention of important aquaculture infectious diseases. Additionally, we seek to enhance spore-based vaccines or SporoVaccines immunogenicity by incorporating molecular adjuvants that target specific immune receptors, thereby amplifying mucosal and systemic immune responses. While adjuvants are well-established in injectable vaccines, their application in oral immunisations remains largely underexplored. By combining these adjuvants with our SporoVaccines, our approach has the potential to improve oral vaccination performance.

## Materials and Methods

To develop the SporoVaccines, key immunogenic proteins from two major bacterial pathogens in aquaculture *(A. salmonicida* and *S. iniae*) were fused to the spore coat protein *CotY*, allowing the antigens to be stably anchored to the spore's surface, ready to interact with the fish's immune system. To try to enhance SporoVaccines efficacy, molecular adjuvants were also fused to a different spore coat protein, *CotZ*. Currently, *in vivo* trials with zebrafish larvae (*Danio rerio*) are being conducted to evaluate vaccine efficacy by testing and comparing different vaccination strategies: (i) spores carrying only antigens; (ii) co-delivery of spores carrying antigens and spores carrying adjuvants; (iii) co-expression of antigens and adjuvants on the same spore.

## Future Perspectives

After identifying the most promising SporoVaccines candidates in the zebrafish trials, the top-performing SporoVaccines will be tested in economically important fish species. European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) will be used as biological model species.

The outcomes of this research are expected to provide a novel and scalable approach to fish vaccination, contributing to disease control in various commercially important aquaculture species. By reducing the need for antibiotics, this work also supports the transition towards a more sustainable, health-conscious, and environmentally friendly aquaculture industry.

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# MATERNAL TRANSCRIPTOME BIOMARKERS FOR EGG QUALITY IN LONGFIN YELLOWTAIL AND PACIFIC RED SNAPPER

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## Introduction

The longfin yellowtail (*Seriola rivoliana*) and the Pacific red snapper (*Lutjanus peru*) are commercially important aquaculture marine species. These species mature and spawn spontaneously in captivity, which represents an advantage in the control of reproduction. However, the egg quality among spawns is variable yet unpredictable, where poor-quality spawns may show low early larval survival and body deformities. Therefore, we investigated differential expression levels of maternal transcripts between poor and good newly fertilized eggs.

## Materials and methods

We collected spawns during 2023 and 2024. Each spawn was collected after ~ two hours of fertilization. We analyzed zootechnical and biochemical measurements and larval survival to distinguish good vs poor spawns. Then, we investigated expression levels of maternal transcripts through RNA-Seq and RT-qPCR. Transcriptome libraries were constructed using NEBNext® Ultra II™ RNA Illumina and mapped to the reference genome of *Seriola dumerili*. We analyzed differential expression, GO and KEGG enrichment analysis, and validated many maternally derived DEGs by RT-qPCR.

## Results

Body abnormalities will lead to deformed and lethargic larvae with low survival (Fig.1A). Poor-quality eggs were characterized by a disruption in Wnt signaling, Tight junction, Oxidative phosphorylation and Ribosome (Fig. 1B).

Larval survival at the onset of exogenous feeding was the only accurate criterion of egg quality. Body abnormalities can be detected from the prehatch stage, leading to deformed and lethargic larvae with low survival. Notably, in *Seriola rivoliana*, poor-quality eggs were characterized by a disruption in protein synthesis (Ribosome), energy production, and the Wnt-signaling pathway. In contrast, we found that in *Lutjanus peru*, poor-quality eggs were only characterized by a disruption in protein synthesis (Ribosome).

## Discussion

Our study agrees with recent research on zebrafish. This study revealed that poor-quality eggs contained an aberrant proteomic profile that lacked proteins involved in protein synthesis and energy and lipid metabolism, implicating a Wnt signaling in this maturation defect that prevents a proper transit through oocyte final maturation (Yilmaz et al. 2017).
Additionally, gene knockdowns of many ribosomal proteins in morphant zebrafish larvae resulted in a specific phenotype, with varying degrees of abnormality in the brain, body trunk, eyes, and ears at about 25 hours post-fertilization (Uechi et al. 2006).

Disrupted ribosome genes such as *rps3a* and *rpl35* could be involved in many body deformities (Uechi et al. 2006).

The *uqcrc2* gene is essential for the electron transport chain in energy production. The downregulation of this gene may lead to suboptimal mitochondrial functioning. In zebrafish, high oocyte ATP levels occur during the G2-M1 transition, where the insulin-like growth factor-1 (IGF1) signaling plays a crucial role in regulating mitochondrial bioenergetics (Biswas et al. 2023).

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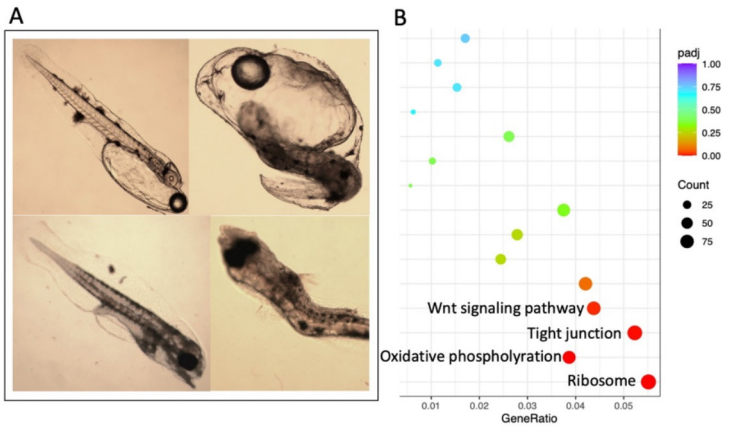


Figure 1A. Healthy hatched (Top left) and mouth opening (Bottom left) larvae. Deformed hatched (Top right) and mouth opening (Bottom right) larvae.
Figure 1B. KEEG enrichment. Red circles were significantly downregulated.

Regarding the Wnt-signaling, it targets cyclin D1(*ccnd1*), regulating the G1 to S phase transition (Röhrs et al. 2009). Frizzled receptors, such as the *fzd7* gene. In *Xenopus laevis*, embryos derived from oocytes depleted of maternal *Xfz7* RNA were deficient in dorsal-anterior structures (Sumanas et al. 2000). During embryonic development in Japanese flounder, *Paralichthys olivaceus,* it was found that *fzd7* was highly expressed during the blastula stage (Hao et al. 2021).
The dickkopf Wnt signaling pathway inhibitor (*dkk1*) gene negatively modulates the Wnt β-catenin pathway. In both *Xenopus* and zebrafish, Dkk1 interacts with the Dally-like homolog Knypek. It regulates gastrulation movements, developing the neural tube and other tissues (Caneparo et al. 2007).

Other genes not disrupted, but essential to assess egg quality, were cyclin B2 *ccnb2* regulating the G2 to M phase transition (Fernández Míguez et al. 2024). The *stk10* gene encodes a serine/threonine kinase with a critical role in oocyte maturation, by acting as a kinase that phosphorylates and activates Plk1, a polo-like kinase (Qian et al. 1998).
Therefore, we found many genes that can be used as biomarkers to assess egg quality and monitor the maternal-to-zygotic transition in this species.

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# CONDITION DEPENDENT EXPRESSION OF MELANIN-BASED SKIN PIGMENTATION IN ATLANTIC SALMON (*Salmo salar*)

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## Introduction

Access to food is a fundamental welfare requirement for farmed fish, directly influencing growth, body condition, and survival. Feed intake and appetite are shaped by environmental factors, social dynamics, and individual variation, making these traits important welfare indicators alongside growth rate and condition (Jobling et al., 2012; Noble et al., 2018). Identifying non-invasive markers of feeding history could provide valuable tools for welfare assessment. Melanin-based traits are linked to condition, stress responsiveness, immune function, and resource-holding potential across vertebrates, suggesting they may act as honest indicators of quality (Roulin & Ducrest, 2011). In Atlantic salmon, more spotted individuals show lower stress reactivity and reduced parasite load (Kittilsen et al., 2012), and in rainbow trout, pigmentation patterns have been genetically linked with faster growth (Rodríguez et al., 2019). Under controlled feeding and nutritional conditions, we test whether variation in feed intake is associated with the development and expression of melanin-based skin pigmentation in juvenile Atlantic salmon.

## Methods

Twelve juvenile Atlantic salmon (control group; 229.7 ± 49.4g, mean ± SD) from a larger experiment were transferred to isolation in 50L compartments within glass aquaria after one year of group rearing (Fig.1A). Fish were hand-fed (max. 1% BW $day^{-1}$) for three weeks to enable detailed monitoring of daily feed intake. Photographs (both sides) and biometric measurements were collected at the start and end of the isolation period to assess changes in melanin-based skin pigmentation and growth. Melanin-based skin spots within the region of interest (ROI; Fig.1B) were manually counted in ImageJ using the "cell counter" plugin, and ROI area was measured to calculate spot density (spots/$cm^2$).

## Results and discussion

All fish displayed stress-induced anorexia immediately after transfer to a novel environment, but feed intake gradually increased throughout the observation period (Fig. 2A). Despite previous studies linking pigmentation patterns to growth in rainbow trout, pre-existing variation in melanin spot density was not predictive of performance (feed intake, growth, and change in K-factor) during acclimation to a novel environment. In contrast, melanin spot pattern development was tightly linked to individual performance. Change in spot density was strongly positively correlated with growth (% BW; Spearman's $\rho = 0.87$, $p < 0.001$; Fig. 2B), total feed intake (% BW; $\rho = 0.86$, $p = 0.001$), and change in condition factor ($\rho = 0.80$, $p = 0.003$), indicating that the development of melanin-based pigmentation patterns closely reflects recent growth and feeding history. These findings highlight spot density and contrast changes as a promising welfare indicator in farmed Atlantic salmon, with potential for integration into automated image or video monitoring systems. Such monitoring could offer insight into recent feeding history and individual condition without additional handling stress, following validation under commercial farming conditions.

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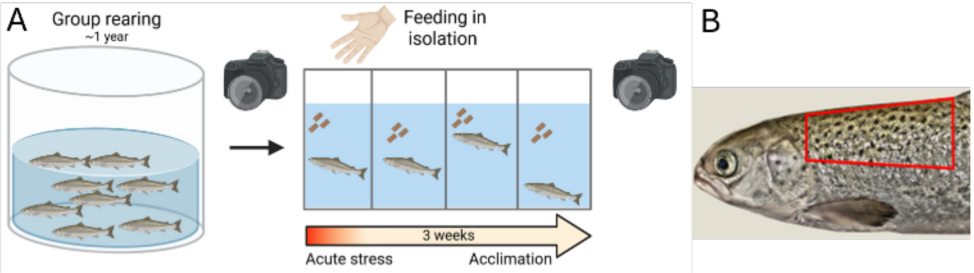


**Fig.1: A)** Overview of experimental design. **B)** Photo illustrating the region of interest (ROI) used for quantification of melanin skin spots. Landmarks of the ROI: end of the gill-lid to the base of the dorsal fin (horizontally) and from the lateral line to the top of the dorsal side (vertically).

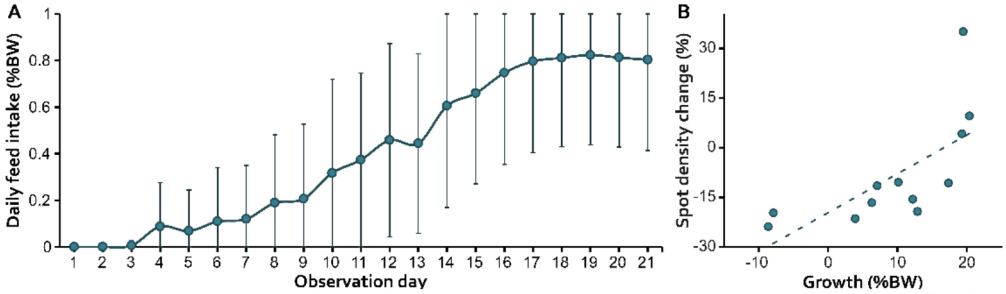


**Fig.2:** Feeding response and pigmentation dynamics in juvenile Atlantic salmon following transfer to isolation**. A)** Average ± SD daily feed intake (% body weight; limit 0-1) over a 21-day period in a novel environment. **B)** Linear relationship between growth (% body weight gain) and change in melanin-based skin spot density. Regression line shows that growth significantly predicted pigmentation change (β = 1.17, 95%CI: [0.29, 2.06], $p$ = 0.014, $R^2$ = 0.47) with higher growth associated with greater retention or development of melanin spots.

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# SPOREFORMING BACTERIA AS A PROBIOTIC IN GILTHEAD SEABREAM *Sparus aurata*: EFFECTS ON GUT HEALTH AND FUNCTIONALITY

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## Introduction

With the expansion of the aquaculture sector and pressure to reduce fishmeal (FM) use, there was an increase in dietary incorporation of plant-feedstuffs (PF). Although PF are a good alternative, they possess non-starch polysaccharides (NSPs) that fish are not able to digest and thus, might compromise fish intestinal integrity and diets digestibility, ultimately affecting fish performance (Sinha et al., 2011). Probiotics are living organisms that upon ingestion in certain amounts exert health benefits beyond inherent basic nutrition (Guarner and Schaafsma, 1998). Among the reported beneficial effects is the capacity to produce enzymes that hydrolyse NSPs and enhancement of host immune status and gut histomorphology (Pérez-Sánchez et al., 2014; Serra et al., 2019). The present study aimed to assess the effect of a novel sporeforming probiotic strain with NSPase (Non-Starch Polysaccharides-active hydrolases) activity incorporated in a PF-based diet, on gilthead seabream gut health and functionality through the measurement of intestinal digestive enzymes, antioxidant and immune-status, and histomorphology.

## Material and Methods

Gilthead seabream juveniles (34g) were fed two isoproteic (47% protein) and isolipidic (17% lipids) diets: one control diet mainly PF-based (blend of soybean, rapeseed and sunflower meal, wheat and corn gluten, soy protein concentrate), with 12.5% fishmeal inclusion, and a diet similar to the control but supplemented with a probiotic *Bacillus* ($2.23x10^9$ CFU $Kg^{-1}$ bacterial isolate (FI99), Serra et al., 2019). Each diet was fed to triplicate groups of fish twice daily until visual satiation, 6 days a week, during 11 weeks. At the end of the trial, 3 fish per tank were sampled 5 hours after the morning meal for collection of intestines for measurement of specific activities of digestive (total alkaline proteases, lipase, α-amylase) and antioxidant (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR)) enzymes and oxidative damage biomarker for lipids (LPO). Intestine of 2 other fish per tank were sampled for expression analysis of immune-related (*interleukin-10* (*il-10*), *interleukin-1-β* (*il-1β*), *cyclooxygenase 2* (*cox*), *immunoglobulin M* (*igM*)) and oxidative stress-related (*heat shock protein 70* (*hsp70*), *cat*, *sod*, *gpx*) genes and for histological processing and morphological evaluation. All data were analysed by one-way ANOVA with a significant level of 0.05 used for the rejection of the null hypothesis.

## Results

Intestinal digestive enzyme activity (total alkaline proteases, lipase, α-amylase), immune-related (*il-1β*, *cox*, *igm*) and oxidative stress-related (*hsp70*, *cat*, *sod*, *gpx*) gene expression, as well as SOD and CAT activities and histomorphology, were not affected by probiotic inclusion. However, *il-10* expression was lower in fish fed the probiotic diet. GPX and GR activities, along with LPO level, are currently being determined.

## Conclusions

Dietary supplementation with the tested probiotic and supplementation level had no major effect in gilthead seabream gut health and functionality. The observed reduction in *il-10* expression suggests that probiotic inclusion modulated the anti-inflammatory response without promoting intestinal inflammation, as pro-inflammatory markers remained unchanged.

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| | Diets | | |
|---|---|---|---|
| | Control | Probiotic | *p* -value |
| Proteases | 810.2 ± 149.3 | 719.9 ± 108.3 | 0.680 |
| Lipase | 12.4 ± 2.85 | 14.1 ± 2.24 | 0.160 |
| Amylase | 567.0 ± 196.9 | 514.1 ± 231.9 | 0.609 |
| *il-10* | 1.25 ± 0.66 | 0.43 ± 0.21 | 0.028 |
| *il-1b* | 1.15 ± 0.41 | 1.07 ± 0.84 | 0.834 |
| *cox* | 1.22 ± 0.62 | 1.38 ± 1.07 | 0.776 |
| *igm* | 2.44 ± 1.78 | 2.27 ± 1.94 | 0.882 |
| *hsp70* | 1.10 ± 0.18 | 0.95 ± 0.43 | 0.456 |
| *cat* | 1.16 ± 0.46 | 0.92 ± 0.47 | 0.401 |
| *sod* | 1.13 ± 0.32 | 0.98 ± 0.58 | 0.610 |
| *gpx* | 1.12 ± 0.30 | 1.18 ± 0.44 | 0.761 |
| SOD | 23.9 ± 9.1 | 24.5 ± 9.20 | 0.913 |
| CAT | 87.6 ± 12.0 | 90.9 ± 27.7 | 0.774 |

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# SPOREFORMING BACTERIA AS A PROBIOTIC IN GILTHEAD SEABREAM *Sparus aurata*: EFFECTS ON GROWTH AND APPETITE REGULATION

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## Introduction

In an aquaculture context, feed intake (FI) assumes high importance, since growth needs to be maximized and conversion of ingested feed into growth increment must be optimized. Control of FI is crucial for proper growth and development of animals and it is under endocrine control. Plant feedstuffs (PF), which are increasingly being used in aquafeeds are rich in non-starch polysaccharides (NSP, undigestible for fish), thus compromising PF nutritional value (Sinha et a., 2011). However, gut microbiota with the capability of producing non-starch polysaccharides degrading enzymes (NSPases) are able of fermenting NSP (Serra et al., 2019). Moreover, the importance of gut bacteria in fish metabolism was already shown (Butt and Volkoff, 2019) and a connection between probiotics use and changes in appetite-related hormones was already established (Guerreiro et al., 2025). Thus, the present study aimed to assess the effect of a novel sporeforming probiotic strain with NSPase (Non-Starch Polysaccharides-active hydrolases) activity incorporated in a PF-based diet, on gilthead seabream growth, FI, feed utilization, plasmatic metabolites, and appetite-related gene expression.

## Material and Methods

Gilthead seabream (*Sparus aurata*) juveniles (34g) were fed two isoproteic (47% protein) and isolipidic (17% lipids) diets: one control diet mainly PF-based (blend of soybean, rapeseed and sunflower meal, wheat and corn gluten, soy protein concentrate), with 12.5% fishmeal inclusion, and a diet similar to the control but supplemented with a probiotic *Bacillus* ($2.23x10^9$ CFU $Kg^{-1}$ bacterial isolate (FI99) Serra et al., 2019). Each diet was fed to triplicate groups of fish twice daily until visual satiation, 6 days a week, during 11 weeks. At the end of the trial, fish in each tank were bulk-weighed after one day of fasting. After the final weighing fish continued to be fed for 3 more days to minimize manipulation stress and then 3 fish per tank were sampled 5 hours after the morning meal for collection of blood from the caudal vein, for plasma metabolites analysis, and of hypothalamus, anterior intestine, stomach and liver for expression analysis of appetite-related (*agouti-related protein* (*agrp*), *neuropeptide Y* (*npy*), *cocaine- and amphetamine-regulated transcript 2* (*cart*), *cholecystokinin* (*cck*), *peptide YY* (*ppy*), *leptin*, *ghrelin*) genes. All data were analysed by one-way ANOVA with a significant level of 0.05 used for the rejection of the null hypothesis.

## Results

Gilthead seabream final body weight, FI, feed efficiency, plasmatic glucose, cholesterol and triacylglycerides, gene expression of hypothalamus *agrp*, *npy* and *cart*, intestine *cck* and *pyy*, liver *leptin*, and stomach *ghrelin* were not affected by dietary probiotic inclusion (Table I).

## Conclusions

Dietary supplementation with the tested probiotic and supplementation level had no effect in gilthead seabream growth performance, FI, feed efficiency, plasmatic metabolites, or appetite-related gene expression. However, there was a tendency toward improved feed efficiency in fish fed the probiotic diet, while the high variability in fish final body weight among fish in the control group prevented the detection of potential growth differences.

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| | Diets | | |
|---|---|---|---|
| | Control | Probiotic | *p* -value |
| Final body weight (g) | 79.3 ± 8.14 | 89.2 ± 2.69 | 0.12 |
| FI (g kg $ABW^{-1}day^{-1}$) | 17.7 ± 1.97 | 18.4 ± 0.95 | 0.36 |
| Feed efficiency | 0.59 ± 0.01 | 0.63 ± 0.02 | 0.06 |
| Glucose | 184.3 ± 24.5 | 178.5 ± 20.4 | 0.60 |
| Cholesterol | 212.3 ± 30.7 | 233.3 ± 29.6 | 0.16 |
| Triacylglycerides | 355.9 ± 91.7 | 368.3 ± 73.4 | 0.75 |
| Hypothalamus *agrp* | 1.33 ± 0.43 | 0.95 ± 0.27 | 0.79 |
| Hypothalamus *npy* | 1.06 ± 0.47 | 1.24 ± 0.57 | 0.53 |
| Hypothalamus *cart* | 1.32 ± 0.61 | 0.75 ± 0.22 | 0.95 |
| Intestine *cck* | 0.96 ± 0.43 | 1.02 ± 0.23 | 0.54 |
| Intestine *pyy* | 0.93 ± 0.47 | 1.04 ± 0.32 | 0.62 |
| Liver *leptin* | 0.27 ± 0.16 | 0.29 ± 0.24 | 0.10 |
| Stomach *ghrelin* | 0.67 ± 0.22 | 0.68 ± 0.40 | 0.44 |

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This project was funded by national funds through FCT – Foundation for Science and Technology under the project FEEDGUTBRAIN (2023.13660.PEX, DOI https://doi.org/10.54499/2023.13660.PEX), and within the scope of the Strategic Funding UIDB/04423/2020 (https://doi.org/10.54499/UIDB/04423/2020), UIDP/04423/2020 (https://doi.org/10.54499/UIDP/04423/2020), and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020). P.G has a PhD scholarship (https://doi.org/10.54499/2021.05635.BD) and I.G. a scientific employment contract (https://doi.org/10.54499/2022.00885.CEECIND/CP1728/CT0013), both supported by FCT.

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Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition – A review. Food Chemistry 127:1409-1426.

# MULTIGENERATIONAL TOXICITY OF CIPROFLOXACIN, SULFAMETHOXAZOLE AND TETRACYCLINE IN *Ceriodaphnia dubia*

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Pharmaceutical pollution in aquatic systems increasingly threatens non-target organisms such as cladocerans. While acute effects of veterinary antibiotics are well documented, little is known about their chronic and multigenerational toxicity, particularly under realistic environmental scenarios. Conventional wastewater treatment plants remove only 32–78% of antibiotics, and even advanced treatments seldom assure complete elimination—for example, sulfamethoxazole removal in membrane bioreactor (MBR) systems was shown to be as low as 6.5%. These concerns are amplified in conflict-affected regions such as Ukraine, where war-related destruction of wastewater infrastructure has led to the discharge of untreated sewage including pharmaceutical residues into freshwater bodies, increasing ecological and public health risks.

In this study, we investigated the multigenerational toxicity of three widely used veterinary antibiotics—ciprofloxacin (CIP), sulfamethoxazole (SMX), and tetracycline (TC)—on the cladoceran *Ceriodaphnia dubia*. A semi-static exposure design was applied across three consecutive generations (F1–F3), with test concentrations ranging from 0.1 to 10 mg/L. Key life-history parameters including survival, reproductive success (offspring per surviving female), total population output, and adult body length were assessed after 21 days of exposure in each generation.

The results revealed strong generation-dependent effects. TC was the most toxic compound, reducing survival to 10–20% in F2 and F3 at 3 mg/L and causing near-complete reproductive failure. CIP significantly suppressed reproduction and population growth beginning at 0.1 mg/L, with 60% mortality observed in F3 at 10 mg/L. SMX showed a biphasic response: at 0.1 mg/L in F2, reproduction was stimulated by ~50%, whereas 5 mg/L led to consistent inhibition of reproduction and reduced body size. SMX had no significant effect on survival at any concentration.

These findings highlight the ecological relevance of low-dose, chronic antibiotic exposure and the potential for lasting impacts on zooplankton populations through multigenerational disruption. The results underscore the importance of integrating long-term biological endpoints into environmental risk assessments, particularly in light of incomplete pharmaceutical removal by wastewater treatment and increasing vulnerability of aquatic ecosystems in regions affected by war or infrastructure collapse.

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# THE IMPORTANCE AND BACKGROUND OF DATA COLLECTION: CASE STUDY FROM HUNGARY

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Freshwater aquaculture plays a vital role in ensuring global food security, livelihood sustainability, and economic development, particularly in developing regions.

However, the sector faces significant challenges related to productivity, ecological services, and market access. A robust data collection framework is essential to address these issues effectively. By systematically gathering, managing, and analysing key data, stakeholders can make informed decisions that enhance operational efficiency and ecological balance. Moreover, standardized data facilitates research, policymaking, and resource allocation, fostering transparency and traceability throughout the aquaculture value chain.

The fisheries Data Collection Framework (DCF) was set up by the European Commission in 2000 to collect, manage and use data in the fisheries sector, to support scientific advice on the Common Fisheries Policy and to monitor and evaluate fisheries activities and resources. In Hungary, the implementation of the tasks of the DCF, as well as further relevant data collections and studies related to the fisheries sector are carried out by the Institute of Agricultural Economics under the Hungarian Fisheries Operational Programme Plus (MAHOP Plusz), co-financed by the Hungarian budget (30%) and the European Maritime, Fisheries and Aquaculture Fund (EMFAF, 70%). As part of this project, the Institute also devotes considerable resources to improving its fisheries data collection system to increase the availability and visibility of data.

The presentation will describe the developments and data collections planned under the Hungarian DCF project, the expected results, and the potential risks and ways to manage them.

These abstract underscores the critical need for implementing structured data collection systems to optimize performance, minimize risks, and support the sustainable development of freshwater aquaculture.

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# ADVANCING AQUACULTURE PERFORMANCE THROUGH NANOBUBBLE TECHNOLOGY: CASE STUDY RESULTS FROM MOLEAER DEPLOYMENTS IN *Salmonid* PRODUCTION

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## Introduction

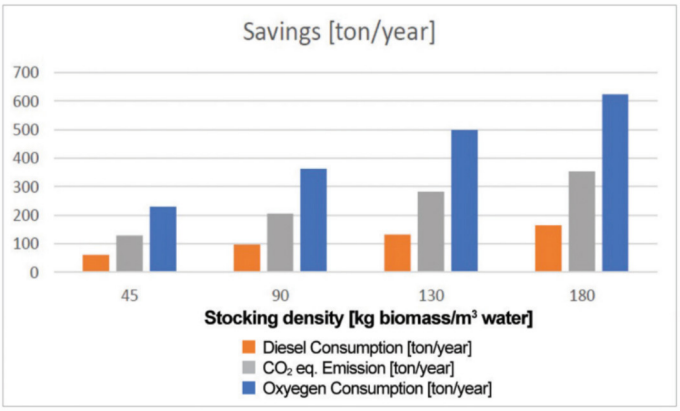
Nanobubble technology, characterized by the generation of gas-filled bubbles under 200 nanometers in diameter, is quickly gaining adoption in aquaculture due to its unique gas-transfer abilities and water-modifying properties. While interest in this technology is growing, successful outcomes depend on precise integration, tailored deployment strategies, and optimization of nanobubble concentration.

Moleaer's nanobubble generators have been tested and validated in diverse aquaculture environments to assess their impact on oxygenation efficiency, water quality, and fish welfare. This abstract synthesizes outcomes from four field case studies: SalMar InnovaNor (waiting cages, Norway), Australis Seafoods (holding tanks, Chile), Lødingen Fisk (RAS Section, Norway) and Rostein (Well boat, Norway). Moleaer's nanobubble solutions were integrated to optimize oxygenation efficiency, water quality and fish welfare.

Nanobubbles differ from conventional macro- and micro-bubbles in both behavior and impact. Their small size and near-neutral buoyancy allow them to remain suspended in water for extended durations, enabling uniform oxygenation without the rapid rise and burst of larger bubbles. Their stability enables more efficient gas dissolution, resulting in higher oxygen transfer efficiency (OTE) and more consistent dissolved oxygen (DO) levels, even in deep zones. Moreover, nanobubbles catalyze localized physical, biological, chemical effects – that support microbial suppression and improved biofilm control. When precisely integrated and optimized, these effects provide a sustainable solution for operators to reduce costs and lower emissions while enhancing fish welfare, water quality, and system performance and efficiency.

## Results

In SalMar InnovaNor's waiting cages at their Senja processing facility in Norway, Moleaer's submersible nanobubble generator was evaluated under varying oxygen demands. The system achieved OTE >85% under all flow conditions, with average DO levels rising from 46.5% to 88%. Importantly, the technology allowed SalMar to maintain optimal DO levels even during summer high-temperature events, enabling increased fish crowding capacity without compromising welfare. Operators reported more rapid and flexible response times, improved stress recovery, and more efficient bleed-out processing.

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At the Dumestre plant of Australis Seafoods in Chile, two nanobubble generators were installed in a 1,000 m³ holding tank. The upgrade reduced oxygen consumption by 63% compared to the previous Venturi-based system and enabled an increase in stocking density from 90 kg/m³ to 120 kg/m³ without compromising fish welfare. Notably, the fish exhibited firmer scales and less flaking, correlating with improved water quality and reduced stress indicators.

Within the first 48 hours of integrating the technology at the start-feeding system RAS section of Lødingen Fisk's in Norway. Over 50 days of continuous use, ammonia removal rates increased by 68%, water turbidity dropped by 30%, and ozone usage for disinfection decreased by 67%. Oxygen Transfer Efficiency exceeded 94%.

Rostein AS, a leading Norwegian well boat operator, integrated Moleaer's solutions to enhance oxygenation efficiency during live fish transport. The implementation resulted in 86% Oxygen Transfer Efficiency (OTE), effectively doubling the efficiency compared to the previous injector system. This translates into an estimated annual oxygen savings ranging from 230 to 360 tons, estimated annual diesel fuel reduction between 60 to 100 tons, and projected reduction in greenhouse gas emissions between 130 to 200 tons per year.

These improvements not only reduced operational costs and environmental impact but also contributed to maintaining optimal fish welfare during transportation.

Nanobubbles enabled better DO stability, reduced reliance on supplemental oxygen, and supported improved fish handling outcomes during stressful periods. These results suggest that nanobubble technology offers a scalable, adaptable solution for improving water quality, fish health, and efficiency across various production stages and system types.

**Conclusion**

The precise and optimized integration of nanobubble technology has consistently demonstrated measurable benefits across four commercial-scale aquaculture operations. These include improved oxygenation efficiency, enhanced biofiltration performance, better fish welfare, alongside reductions in resource consumption and operational costs.

Moleaer's case studies highlight the versatility of nanobubble solutions across multiple aquaculture applications including RAS, waiting cages, and wellboats. As the industry pursues scalable strategies for sustainable growth, nanobubble technology offers a scientifically backed solution to enhance production efficiency and environmental resilience. Ongoing research will continue to refine the mechanisms and expand applications across additional species and system types.

# BIO-BASED ELECTROCHEMICAL SYSTEM FOR EVALUATING ECOTOXICOLOGICAL THREATS IN THE MARINE ENVIRONMENT

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The integrity of marine ecosystems is increasingly threatened by anthropogenic pollutants, particularly pesticides such as herbicides, fungicides, and insecticides that enter coastal and open ocean waters through agricultural runoff and atmospheric deposition. These substances, especially organophosphorus and carbamate insecticides, can exert toxic effects at trace levels, disrupting aquatic food chains, harming marine biodiversity, and affecting biochemical pathways in organisms.[1,2]induced by various inhibitors, leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission. Hence, acetylcholinesterase inhibitors, interacting with the enzyme as their primary target, are applied as relevant drugs and toxins. This review presents an overview of toxicology and pharmacology of reversible and irreversible acetylcholinesterase inactivating compounds. In the case of reversible inhibitors being commonly applied in neurodegenerative disorders treatment, special attention is paid to currently approved drugs (donepezil, rivastigmine and galantamine Among the biological targets most affected is acetylcholinesterase (AChE, EC 3.1.1.7), an enzyme critical for neural function in both vertebrates and invertebrates.

Given the ecological importance of maintaining marine health and the increasing concern over pollutant accumulation, it is essential to develop monitoring tools that are both sensitive and adaptable to in-situ conditions. Traditional analytical methods, such as HPLC,[3] capillary electrophoresis (CE),[4] or colorimetry,[5]homogenates, cell suspensions, etc., has been described. The enzyme activity is measured by following the increase of yellow color produced from thiocholine when it reacts with dithiobisnitrobenzoate ion. It is based on coupling of these reactions: {A figure is presented} The latter reaction is rapid and the assay is sensitive (i.e. a 10 µ1 sample of blood is adequate though effective, are limited by the need for sophisticated instrumentation, extensive sample preparation, and operation in centralized laboratories. In contrast, biosensors offer significant advantages for environmental monitoring, including simplicity, portability, low cost, high specificity, and the potential for real-time detection.[6]there is a lack of monitoring of these contaminants. Traditional chromatographic methods - high-performance liquid chromatography, capillary electrophoresis, and mass spectrometry - are effective for the analysis of pesticides in the environment but have certain limitations such as complexity, time-consuming sample preparation, and the requirement of expensive apparatus and trained persons to operate. Over the past decades, acetylcholinesterase (AChE

In this work, we present the design and development of an electrochemical biosensor specifically tailored to detect neurotoxic pesticides in seawater via indirect monitoring of AChE activity. The strategy is based on the enzymatic hydrolysis of *p*-acetoxyphenol, a non-electroactive substrate, into hydroquinone, which is electrochemically active and easily detected using cyclic voltammetry.[7] The core of the biosensor consists of AChE immobilized within a stable sol-gel silica matrix, a material that ensures enzyme retention, biocompatibility, and optimal catalytic conditions in the marine environment.

Kinetic studies confirmed the enzymatic hydrolysis reaction, while inhibition experiments using malathion demonstrated a clear, concentration-dependent reduction in enzymatic activity. These findings support the sensor's capability to quantify pollutant levels based on the degree of AChE inhibition, offering a bio-relevant and functional measurement of ecotoxic risk.

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To enhance its applicability in real-world conditions, the AChE-based sensor has been integrated into a custom-designed microfluidic cell. This configuration allows precise control of small sample volumes, minimizes reagent use, and enables continuous monitoring. Moreover, the compact and modular nature of the microfluidic platform facilitates its incorporation into autonomous marine robotic systems. Our vision is to deploy this biosensor as part of a marine robot equipped for in-situ detection of neurotoxic contaminants, enabling early-warning monitoring of water quality and supporting sustainable ocean management.

This research will contribute to the development of next-generation biosensing technologies for marine environmental monitoring, aligning with global efforts to preserve ocean health and biodiversity.

Acknowledgements
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# SUSFEED - SUSTAINABLE FEED PRODUCTION FROM NORWEGIAN BIO-RESOURCES FOR LIVESTOCK AND AQUACULTURE

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## Introduction

Feed production relies on large amounts of bulk proteins, carbohydrates and oils, sometimes produced in remote areas and transported around the globe (Cottrell et al. 2020). Norway's current bioeconomy strategy emphasises the need to increase animal feed production based on domestic ingredientsto strengthen food security, increase sustainability and develop more climate friendly production. This requires a holistic system perspective that looks across multiple different potential sources of animal feed – both those available now, and those likely to become available in near future through the deployment of new raw materials and technologies. The mission of the SUSFEED project is to contribute to enhancing sustainability in Norwegian agriculture and aquaculture, business development and increased food security in Norway. The project aims to develop knowledge about how biological resources for feed production can be produced, distributed and used in a way that contributes to the development of sustainable and high-value agricultural and aquacultural sectors.

There are inadequate analyses of most new applications of novel bioresources for feed ingredients, as they are currently not included in commercial value chains. To address this challenge, we carried out four case studies, selected according to the following criteria: a) they are new applications of bioresources for feed; b) they represent different categories of bioresources (soil, wood, ocean, insect); c) they open up value chains across established sectors (agriculture, forestry, aquaculture); d) they are of interest to study at different stages in a potential value chain; and e) they are of commercial interest among companies. Grass proteins has a valuable amino acid profile comparable to soy proteins (Damborg et al., 2020), but the high fibre content need to be reduced before it can be digested properly by monogastric animals. Wood fibres can be made available in large amounts but is indigestible "as is" for most animals, however, fungal depolymerisation can be applied to improve digestibility. Microalgae provide proteins with optimal amino acid profile for fish and essential n-3-oils, but bioavailability is low. Insects are an established source of feed ingredients, but the nutritional profile of insect derived feed ingredients when reared on different substrates is poorly documented, therefore, the use of alternative feed stocks and their effects on the nutritional value of insects is important to understand. The aim of this study was to characterise these novel feed ingredients and their suitability in feed to identify opportunities and limitations in industrial upscaling and business development of new feed resource value chains.

## Materials and methods

**Case 1 – Grass:** Two different grass mixtures were harvested at different times over the season, characterised and fractionated into protein sediments, fibres and "brown juice" (GEP; Grass Protein Effluents). Grass proteins were used in a feeding trial on Atlantic salmon (*Salmo salar* L.), grass fibres were tested in feed for insects (Case 3), and the GEP was used as growth medium for microalgae cultivation (Case 4).

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**Case 2 – Wood:** Different wood (*Picea abies* and *Betula pubescens*) and fungal species (brown rot fungi: *Gloeophyllum trabeum, Fomitopsis pinicola, Fomotopsis rosea,* and white rot fungi: *Trametes versicolor, Phlebia centrifuga, Plaurotus ostreatus*) were subjected to different processing techniques and tested in insect feeding trials (Case 3) in combination with grass fibres (Case 1).

**Case 3 – Insects:** Grass fibres and fungal depolymerized wood chips were used in feeding trials for mealworms (*Tenebrio molitor*), house crickets (*Acheta domesticus*) and black solder fly larvae (*Hermetia illucens*). Further on, insect meal was used in two different feeding trials on Atlantic salmon and poultry. Insect frass, a co-product of insect production consisting of a mix of shed exoskeletons, droppings, and leftover feed, rich in essential nutrients and microminerals, was used as growth medium for cultivating microalgae (Case 4).

**Case 4 – Microalgae:** Non-axenic cultures of *Chlorella vulgaris, Chlorella sorokinana, Phaeodactylum tricornutum* and *Tetraselmis suecica* were cultivated to test the suitability of insect frass extract (IFE) and grass processing effluent (GPE) as microalgae growth mediums.

**Results and discussion**

Wood particles should be finely ground, significantly depolymerised by fungi and mixed with a feedstock that keeps the moisture in the feed to be used as insect feed. Similarly, grass fibres should also be processed into smaller particles and mixed with some other nutritional feed stock to be attractive as feed for different insects and maintain its moisture. Moreover, it appears that black soldier fly larvae and mealworms are more positive to the incorporation of depolymerised wood and grass fibres compared to house crickets. The chemical composition of GPE and IFE revealed that both solutions should be able to support microalgae growth, if certain microelements are added in bioavailable forms. However, we observed inhibitory growth effects of the GPE extract causing low yields and/or prolonged lag phases which warrants further investigation. Results from the feeding trials on Atlantic salmon and poultry are pending and will be presented.

**Acknowledgements**

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# DEVELOPMENT OF AN ECOSYSTEM SERVICE-BASED ENVIRONMENTAL PERFORMANCE INDICATOR FOR INLAND POND AQUACULTURE

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**Introduction**

The general concept of sustainability and the components of sustainable development has now been well defined (FAO 1989). Sustainability is also one of the most important horizontal aspects of the European Green Deal's priorities. It means such transition in farm systems that is better adapted to environmental conditions, minimises environmental footprints, and preserves natural values. Beyond the formulation of the sustainability goals, to determine the environmental performance of the production systems developing sustainability metrics became more and more urgent. The evaluation of the environmental interactions of aquaculture technologies is also a key component in enhancing sustainability, a principle that is well reflected in the EU aquaculture policies such as Aquaculture Strategic Guideline and Aquaculture Assistance Mechanism.

Filling this knowledge gap wide range of indicator-based evaluations were developed in the latest year (FEAP 2022, Garlock et al. 2024, EC 2024, Nielsen et al. 2025). The aim of these assessments to measure performance of aquaculture production systems in the three key dimensions of sustainability: environmental, economic, community dimensions and comparing aquaculture management systems as well as evaluating the effectiveness of investment and reforms (Garlock et al. 2024).

Our analysis of the current indicators revealed that these methods basically focus for one-way industrial processes and not fully appropriate for evaluating circular production systems. Furthermore, when such circular systems are characterised using these indicators, the results are typically false and significantly lower than those obtained using a natural science-based approach (Knösche et al. 2004, Koushik et al 2020).

**Conceptual framework**

Pond farming in the EU concentrated in the Central Eastern European (CEE) region giving the vast majority of aquaculture production in these countries and being an important part of European agricultural heritage. The pond aquaculture sector produces yearly 80,000 tonnes of carp that is 26 percent of the total EU freshwater aquaculture production in volume and 260 million euros in value (EUMOFA, 2021). Pond farming applying circular approach is operated in a traditional, extensive production system. It is based on natural nutrient cycle and operates as an open ecological system, where natural and technological processes support each other (Halasi-Kovács 2022). It is a well-documented fact that extensive pond aquaculture provides a wide range of ecosystem services in addition to primary fish production (Willot et al. 2019, Palásti et al 2020, Sharma et al. 2024).

A comprehensive assessment of these services has not been conducted yet, and there is an absence of specific environmental performance indicator based on the evaluation of these systems. As a first step in formulating our evaluation system, the concept of ecosystem services (Haines-Young 2023) can provide a natural theoretical framework. However, being developed an indicator, we were facing different long-standing problem: (1) the difficulty of the quantification of ecosystem services; (2) confusion of terms. So, we modified the conceptual framework of ES by adding a new benefit category, the 'environmental benefits' that can well reflect the clear difference between the term of ecosystem service and environmental benefit. Furthermore, it provides an operational solution for developing ecosystem service-based environmental performance indicator that can be integrated in Environmental Impact Assessment (EIA) and Life Cycle Assessment (LCA) studies of fishponds.

**Next steps and results**

In the next step, the ecosystem services provided by fishponds will be assessed. Subsequently, the environmental benefits resulting from these services will be determined and evaluated. Ecosystem service-based environmental performance indicator will be developed and scaled based on the environmental benefits of pond aquaculture.

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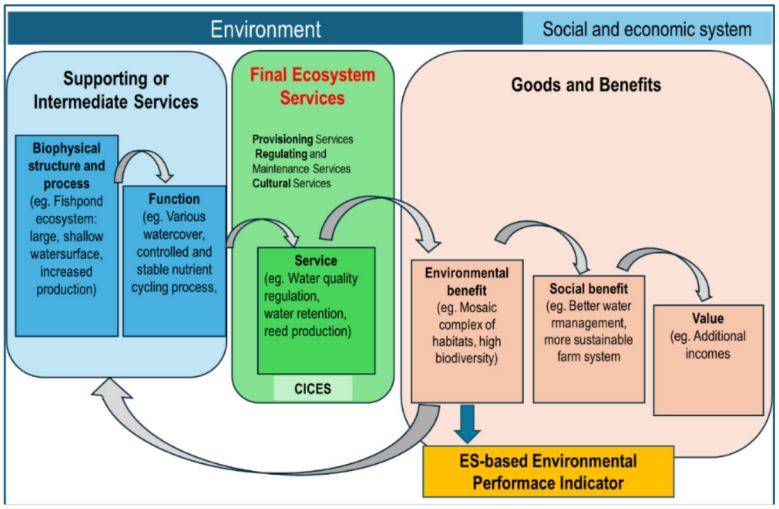
**Figure 1.** Conceptual framework of the ecosystem-based environmental performance indicator




after Haines-Young and Potschin (2023)

The results of this research provide a clear concept and definitions of the ecosystem service-based environmental performance indicator and can provide tools for the evidence-based environmental evaluation of different circular approached extensive, low-trophic aquaculture systems.

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# THE MICROALGAE *Nannochloropsis* FOR UPCYCLING BREWERY WASTEWATER INTO FISH FEEDS: FROM BENCH TO PILOT SCALE CULTIVATION TO FISH TRIALS

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## Introduction

Microalgae offer a sustainable alternative to conventional, chemical-intensive wastewater treatment by harnessing solar energy and converting excess nitrogen and phosphorus from wastewater into bioactive compounds that can be developed into valuable bioproducts (e.g. aquaculture feed). Among various microalgae, *Nannochloropsis oceanica* is particularly appealing due to its robust growth and high lipid content, especially omega-3 polyunsaturated fatty acids (e.g. C20:5n3 or Eicosapentaenoic acid EPA), a critical ingredient for the promotion of growth and immune development in farmed fish. However, scaling up microalgae-based wastewater treatment from laboratory to industrial scale remains a key challenge due to difficulty in maintaining tightly controlled environmental conditions at large scale. This study investigated how different nutrient supply modes (Single-Batch where nutrients are supplied at once at the beginning of cultivation cycle and Fed-Batch where nutrients are periodically replenished throughout cultivation cycle) affect microalgal performance in treating brewery wastewater and how feeding regime can be optimised when upscaling lab production to pilot-scale. The resultant microalgal biomass obtained from brewery-wastewater cultivation was then incorporated into feed formulations for seabream trials. The effect of *Nannochloropsis* biomass inclusion on fish growth and tissue health was assessed. Overall, our study demonstrates the potential of using *Nannochloropsis* to upcycle brewery waste into aquaculture feed, creating a circular food system which contributes to waste management sustainability in the brewing sector and alleviating dependence of farm fishing on wild-caught fish.

## Materials and methods

Raw brewery wastewater was collected onsite from a local brewer in Dublin, Ireland. A total of 4200 L of brewery wastewater were collected from February 2023 to October 2023. The microalgae *Nannochloropsis oceanica* (CCAP 849/10), obtained from Culture Collection of Algae and Protozoa (CCAP, SAMS Ltd., Oban, Scotland), were cultivated on the brewery wastewater. Two different cultivation regimes (Single-Batch and Fed-Batch) were implemented to evaluate the growth sensitivity of microalgae against scaling up the cultivation system, from bench scale (2L flasks and 10L carboys) to pilot scale (900 L PBR). Key nutrients in the wastewater (e.g. nitrate, phosphate) were continuously tracked to assess bioremediation efficiency The harvested biomass in PBR was also analysed in terms of lipid content, fatty acids profile, amino acids, and protein.

For seabream feeding trials, the harvested microalgal biomass from pilot-scale cultivation was then used to partially replace conventional protein and lipid sources in four isoproteic (48%) and isolipidic (18%) experimental diets, formulated to compare against commercial fishmeal-based feeds. A total of 840 fish with an average body weight of 11 g in 12 x 500 L tanks were fed with one of the four experimental diets for 96 days from April to July 2024 (70 fish per tank; 3 tanks per diet). Fish were weighed during the feeding period. At the end of the trial, the fish were randomly selected, anesthetized, and euthanized for biometric and organ index analyses, including liver and viscera weights to calculate hepatosomatic (HSI) and viscerosomatic (VSI).

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**Conclusions**

*Nannochloropsis* was successfully cultivated on brewery wastewater and systematically scaled up from bench to pilot scale. Under bench scale conditions, both cultivation modes achieved a maximum biomass concentration exceeding 1.2 g/L. However, in pilot scale, the early depletion of the nitrogen source under Single-Batch feeding regime resulted in algal growth stagnation and eventually bacterial takeover. In contrast, the Fed-Batch mode enabled continuous and stable biomass production up to 22 days, attaining a maximum biomass concentration of 1.31 g/L. Additionally, the mode of nutrient supply also affecteed microalgae composition and associated features relevant to their potential use as aquaculture feed. The Fed-Batch harvested biomass contained more protein (29.4% vs. 15.1% in biomass) and lipid (44.0% vs. 30.6% in biomass) compared to the Single-batch. In terms of fatty acid composition, Fed-Batch biomass is also enriched in monounsaturated fatty acids (39.6% vs. 29.5% in total fatty acids) and polyunsaturated fatty acids (13.7% vs. 11.7% in total fatty acids) relative to the Single-Batch biomass.

During the seabream trials, feeding *Nannochloropsis* biomass obtained from brewery waste cultivation as a protein and lipid replacement resulted in growth performance similar to a commercial fishmeal-based diets. Additionally, the histopathology of the liver and intestine cells in the fish highlighted that a high replacement of fishmeal and fish oil with *Nannochloropsis* did not trigger any apparent tissue damage and/or malfunction. These results are the first to demonstrate the potential of *Nannochloropsis* biomass cultivated on waste as a viable alternative source of lipids and protein in aquafeeds.

# PHOSPHORUS NUTRITION IN WHITELEG SHRIMP *Litopenaeus vannamei*: CURRENT KNOWLEDGE AND RESEARCH GAPS

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Over the past two decades, the aquaculture production of whiteleg shrimp (*Litopenaeus vannamei*) has increased by 623%, reflecting the species' growing importance in global aquaculture. FAO reports indicate that global production of whiteleg shrimp has surpassed seven million tons per year, with China and Ecuador emerging as the primary producing countries. As market demand continues to rise, shrimp production is projected to exceed current margins, prompting the feed industry to place greater emphasis on refining nutritional strategies for this economically important species. Phosphorus (P) is an essential mineral in aquafeeds, playing a critical role in energy metabolism, nucleic acid synthesis, skeletal development, and exoskeleton in crustaceans. In whiteleg shrimp, efficient phosphorus nutrition is particularly important due to their fast growth rates, low tolerance to P deficiency, and the ecological implications of P leaching in intensive/extensive farming systems. Over the past two decades, considerable progress has been made in defining dietary phosphorus requirements, identifying available phosphorus sources, and improving assessment methodologies—including apparent digestibility studies and predictive modeling. However, major gaps remain, particularly in understanding phosphorus bioavailability from alternative and plant-based ingredients, the interaction of dietary P with other minerals, and the implications of high dietary P levels on shrimp health and water quality. Moreover, standardization of digestibility methods, especially under varying salinity and environmental conditions, remains a key challenge. This review synthesizes current knowledge on phosphorus nutrition in *L. vannamei*, identifies critical research gaps, and highlights opportunities for advancing precision nutrition and sustainability in shrimp aquaculture.

# OFFSHORE AQUACULTURE – TECHNICAL DEVELOPMENT OF A COMBINED AQUACULTURE PROCESS WITH FISH AND MUSSELS FOR THE COASTEL WATERS OF MECKLENBURG – VORPOMMERN

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The purpose of the project is to test various methods of fish and mussel aquaculture and to evaluate their economic viability and environmental compatibility in order to enable the most sustainable, environmentally friendly and local aquaculture production possible in the western Baltic Sea of Mecklenburg-Vorpommern. The fish aquaculture project aims to build and test net-carrier systems for fish farming (*Oncorhynchus mykiss*) that are adapted to the conditions of the shallow and dynamic coastal waters of Mecklenburg-Vorpommern. Two different farming systems with the type names "The Cage" and "Bjørn" are to be built:

"The Cage" is an experimental tiny enclosure for scientific works with a small number of fish (up to 2000). The net is fixed in a static pipe structure which is octagonal cylindric and has a volume of approx. 100 $m^3$. The tubular structure is braced over 4 corners centrally in a plastic pen and sits on a buoyancy frame. This is used for stabilization as well as lifting and lowering. Primarily, it is a test system whose focus is on evaluating the fattening of smolts weighing approx. 100 g to approx. 400 g in approx. 3 months. The goal is to continuously produce portion fish for catering or sale over the masting season of approximately 9 months (April - December).

"Bjørn", on the other hand, is an innovative aquaculture design that is to be evaluated for the practical and fundamental commercial applicability of small-scale systems. For this purpose, a small-sized business (SMB), preferably a regional fishing company, will take over the management. The Institute of Fisheries (IfF) will provide the enclosure, feed, trout and advice. The SMB decides on the stocking density and masting phases in agreement with the IfF.

In addition to the technical and economic issues, accompanying environmental monitoring is being considered, in order to draw conclusions about operational emissions and potential environmental impact. These are of particular importance with regard to the permissibility of such facilities. Due to various requirements of the Water Framework Directive, compensatory measures must be taken to counteract the deterioration of water quality around the cage. The extraction of mussels can be seen as a removal of the nutrients (especially nitrogen and phosphorus) released through fish aquaculture. In parallel to compensating for the fish aquaculture, this can also generate an additional source of income.

Three different mussel culture systems are set up. Surface double longline, surface close single longline and submerged single longline are equipped with data loggers placed at different depths to monitor the movement of the collectors in x, y and z direction. These data loggers are used to test in which system less movement is detected in order to minimize the loss of mussels. In parallel to hydrodynamics, it should also include the possible minimization of predation pressure by birds. In addition, an Acoustic Doppler Current Profiler (ADCP) is installed on the seabed to monitor the currents around the aquaculture facilities and an Automatic Profiling Buoy (APB), which is designed for automatic Conductivity Temperature and Depth (CTD) profiling. The CTD is an instrument with multi parameter functionality with sensors like Oxygen, Turbidity, fluorescence and photosynthetic light sensor. This means that a profile of the water quality can be obtained throughout the year. In addition to the annual profiles, mussels and fish are sampled and analysed for pollutants in the laboratory.

# SHIFTING PRODUCTION CYCLE FROM SEA TO RAS – WHAT DOES THE FISH SAY?

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**Introduction**

In combined RAS (recirculating aquaculture system) and sea cage farming, fish are first farmed in RAS and then transferred to sea cages for the rest of their lives. By increasing the size of fish in RAS, the production cycle of farming can be accelerated, and the sea cage period can be shortened (Bjørndal and Tusvik, 2017). A shorter sea cage period provides means to capture nutrients more efficiently and reduces biological risks (Bjørndal and Tusvik, 2017). Shorter sea cage period would also make it possible to avoid risks associated with overwintering of fish (Donaldson et al., 2008). However, RAS and sea cage farming are very different production methods and the effects of combining them on fish growth and welfare are not well understood. In this study, we investigated 1) Does growth of rainbow trout (*Oncorhynchus mykiss*) farmed in RAS and PRAS (partial RAS) differ after transfer to freshwater and brackish water conditions? 2) Does the size of rainbow trout and/or the timing of transfers affect the growth of rainbow trout? and 3) Does the water quality of initial farming influences the success of transfers?

**Materials and methods**

Rainbow trout were first farmed in RAS and PRAS. In summer, some fish were transferred to fresh and brackish water, while the rest remained in the original RAS and PRAS tanks. The fish were farmed in the fresh and brackish water for five weeks (the summer period). In autumn, another transfer took place, and the fish were again transferred to the fresh and brackish water. The autumn period took also five weeks. The fish were sampled after the summer and autumn periods. The sample fish were killed by an overdose of anaesthetic. The total length, weight and gutted weight were measured. Based on the fish growth data, feed conversion ratio, condition factor, specific growth rate, and thermal-unit growth rate were calculated. A blood samples were taken for haematocrit, plasma osmolality, plasma chloride content, and plasma cortisol content determination.

**Results**

During the summer period, the RAS-farmed fish grew better in the freshwater than in the RAS environment (SGR 34%, TGC 37%, $p < 0.001$) or in the brackish water (SGR 27%, $p < 0.001$). The RAS-farmed fish grew better in the RAS environment than in the brackish water (TGC 30%, $p < 0.001$). The PRAS-farmed fish grew better in the freshwater than in the PRAS environment (SGR 8%, $p < 0.05$) or in the brackish water (SGR 8%, TGC 35%, $p < 0.001$). The PRAS-farmed fish grew better in the PRAS environment than in the brackish water (TGC 33%, $p < 0.001$). In the RAS environments, the PRAS-farmed fish grew better than the RAS-farmed fish (SGR 22%, TGC 37%, $p < 0.001$). In the freshwater, there was no significant difference between the RAS- and PRAS-farmed fish. In the brackish water, the RAS-farmed fish grew better than the PRAS-farmed fish (9%, $p<0.05$).

During the autumn period, the RAS-farmed fish grew better in the RAS environment (SGR 295%, TGC 283%, $p < 0.001$) and in the freshwater (SGR 270%, TGC 333%, $p < 0.001$) than in the brackish water. There was no difference in the RAS-farmed fish between the RAS environment and the freshwater. The PRAS-farmed fish grew better in the PRAS environment than in the freshwater (32%, $p < 0.01$). The PRAS-farmed fish grew better in the PRAS environment than in the brackish water. In the PRAS environment the fish continued to grow normally (SGR 1.39, TGC 0.23), while in brackish water fish did not eat and lost weight (SGR -0.07, TGC -0.01). PRAS-farmed fish grew also better in the fresh than in the brackish water. In the freshwater, the fish continued to grow (SGR 1.05, TGC 0.23), while in the brackish water the fish did not eat and lost weight (SGR -0.07, TGC -0.01). In the RAS environments, there were no difference between the RAS- and PRAS-farmed fish. In the freshwater, the RAS-farmed fish grew better than the PRAS-farmed fish (29%, $p < 0.01$). In the brackish water, the RAS-farmed fish grew better than the PRAS-farmed fish. The RAS-farmed fish continued to grow (SGR 0.37, TGC 0.06), while the PRAS-farmed fish did not eat and lost weight (SGR -0.07, TGC -0.01).

*(Continued on next page)*

**Discussion**

Seawater transfer plays an important role in salmonid production, where juveniles are produced on land in freshwater and then transferred to the sea for further farming (Thorstad et al., 2012; van Rijn et al., 2021). This study investigated the transfer of two different sizes of rainbow trout from two different RAS environments to fresh and brackish water. We observed differences in the growth of RAS- and PRAS-farmed fish after transfers to the fresh and brackish water. The most striking exception was the results for the PRAS-farmed fish in the brackish water during the autumn period. The fish did not eat despite continuous offering of the feed and therefore lost weight. Stress does not seem to explain the differences in appetite and growth of fish, as we did not observe significant differences with a clear pattern in the physiological parameters. Our results also show that the size of fish and the timing of transfer affect growth of fish. In this study, the smaller fish transferred in the summer grew better than the larger fish transferred in the autumn. Based on our study, the environment and water quality in the initial farming did not seem to affect growth of fish after transfer in themselves. Continuous lighting and feeding, as well as the fish growth in the RAS and PRAS environments, may influence the growth success in the further farming. Ultimate reasons for these phenomena remain unclear and need clarification to develop the farming method for wider use in salmonid aquaculture.

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# EFFICACY OF NOVEL SETTLEMENT SUBSTRATES FOR STANDARDISING SETTLEMENT FOR COMMERCIAL PRODUCTION OF ABALONE AND SEA URCHINS

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## Introduction

Inconsistent settlement rates for commercially important species remain a bottleneck at the beginning of the production value chain. The commercially important species such as sea urchin *Paracentrotus lividus,* and abalone *Hailotitos discus hannai* & *H. midae* are increasing in commercial interest for aquaculture in Europe and South Africa.

However suitable larval settlement substrates (e.g. *Ulvella lens*) remain untested and in many commercial settings. Inconsistent low settlement rates are currently achieved at the nursery stage by commercial hatcheries and efforts to establish other commercial hatcheries around Europe, have been hampered by inconsistencies in production, which remain a key bottleneck (Hannon et. al., 2013, Lawrence and Lawrence, 2014).

Development of settlement cues and production methods by the Hannon et. al., (2014) have been transferred from Ireland to Spain (2015) and South Africa (2017) to support hatchery production of sea urchins and abalone under commercial conditions. This presentation is evaluating the efficacy and implementation of the use of novel settlement cues and how they can be used to standardise settlement with a guaranteed average settlement range of 50-70% inside 24-hours from transfer to settlement tanks or systems.

Under commercial conditions postlarval survival increases significantly once an effective settlement cue is utilised with an overall increase of 30-40% hatchery output for the same time period.

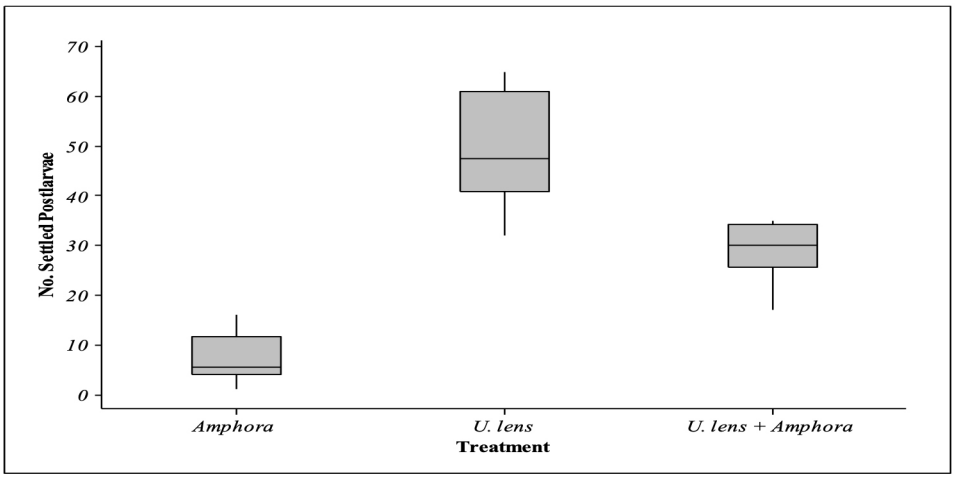


Figure 1. Box-Plot comparing the settlement achieved on the different treated settlement substrates. There is a significant statistical difference between the settlement rates of each treatment with the *U. lens* treatment achieving the highest number of settled post larvae ($P < 0.05$)

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**Materials & Methods**

Overcoming the production barriers for the culture of *Ulvella lens* required the streamlining of the production method to aid the tech transferability to commercial production. This required the removal of the trial and error approach. All cultures were establish under commercial conditions at commercial facilities in Ireland, Spain and South Africa.

Cultures of *U. lens* were isolated and scaled up in culture to increase available adult sporophyte biomass for mass sporulation which can be repeated every 14-days once the sporulation cycle is initiated. This has halved the current time from the original method developed in the 1980s'. Post larval settlement and survival was assessed along with their feeding transitions and weaning

**Results**

Significant differences were observed when the cues were assessed at commercial scale when compared to the benthic diatoms (e.g. *Amphora* Spp.). Using monospecific diatoms have been shown to increase settlement rates than relaying on natural assemblages of wild benthic diatoms available to hatcheries. When *U. lens* is used in commercial production we have seen an average settlement rate of 50-70% inside the first 24-hours after the competent larvae are introduced to the settlement cue (Figure 1).

**Discussion**

*U.* lens can be used as a settlement cue for sea urchins, abalone and sea cucumbers and is ready for transfer into commercial production of low trophic aquaculture species in the hatchery.

knowledge transfer resulted in a fully scaled *U. lens* hatchery directly improved spat production by 30% to 40% for abalone hatcheries and resulted in a on average settlement of 50-70% for sea urchins under commercial conditions.

Hatcheries are very complex and the *U. lens* used in the settlement phase gave the species produced a head start in life; and not only did settlement production improve, but so did weaning survival as the macroalgae phase of the mollusc was already achieved in settlement, and sea urchins are able to graze as postlarvae with no feeding transition period or loss in growth rates.

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# NEW ROTIFER ENRICHMENT IMPROVES GROWTH IN LARVAL ATLANTIC COD (*Gadus morhua*)

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## Introduction

Early growth and nutrition in cod larval farming significantly affect weight development during later stages, impacting the overall performance of farmed fish (Hansen et al., 2018). Renewed interest in cod farming has emerged due to advancements in selective breeding and declining wild stocks (Puvanendran et al., 2022). To further develop the industry, new enrichment products for rotifers have been tested in cod production.

## Materials and Methods

Eggs and sperm were stripped from broodstock and fertilized using the standard protocol at the CMA. Five families were created, and fertilized eggs from each family were incubated in 25L conical silo incubators with a flow-through of ambient water at 4-5°C. After 100% hatch, 3,000 larvae from each family were transferred to one of four replicates first feeding tanks (190L) per treatment (15,000 larvae per tank), with water temperature gradually increased from 4-5°C to 10°C over 5-10 days (~1°C per day).

## Feeding Protocol

Rotifers were enriched with three different enrichments:

1. Control group – Rotifers enriched with Multigain®, PhosphoNorse®, Pavlova, Chlorella.
2. Treatment 1 – Rotifers enriched with new enrichment formula I.
3. Treatment 2 – Rotifers enriched with new enrichment formula II.

**Table 1: Details of the feeding protocol used in this experiment.**

| Age (DPH) | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Algae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| # of feedings/d | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ml/d | 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Rotifers | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| # of feedings/d | 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| million/day | 3.05 | | | 5.25 | | | | | | | | | | | | | | | | | | | | | 3.8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| million/feeding | 0.61 | | | 1.05 | | | | | | | | | | | | | | | | | | | | | 0.76 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Artemia | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| # of feedings/d | | | | | | | | | | | | | | | | | | | | | | | | | 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | | | | |
| million/day | | | | | | | | | | | | | | | | | | | | | | | | | 0.85 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2.65 | | | | |
| million/feeding | | | | | | | | | | | | | | | | | | | | | | | | | 0.17 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.53 | | | | |
| Dry feed | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| # of feedings/d | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | | | | | | | | | | | | | | | | | 6 | | | | |
| gram/day | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1.2 | | | 2.4 | | | 3.6 | | | | | | | | | 12 | | | | |
| gram/feeding | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.2 | | | 0.4 | | | 0.6 | | | | | | | | | 2 | | | | |
| Flow rate | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| % / day | 800 | | | | | | | | | | | 880 | | | | | | | | | | | | | 960 | | | | | 1400 | | | | | | | | | 1600 | | | | | | | | | | | | | 2000 | | | | | | 2400 |
| L / min | 1 | | | | | | | | | | | 1.1 | | | | | | | | | | | | | 1.2 | | | | | 1.75 | | | | | | | | | 2 | | | | | | | | | | | | | 2.5 | | | | | | 3 |

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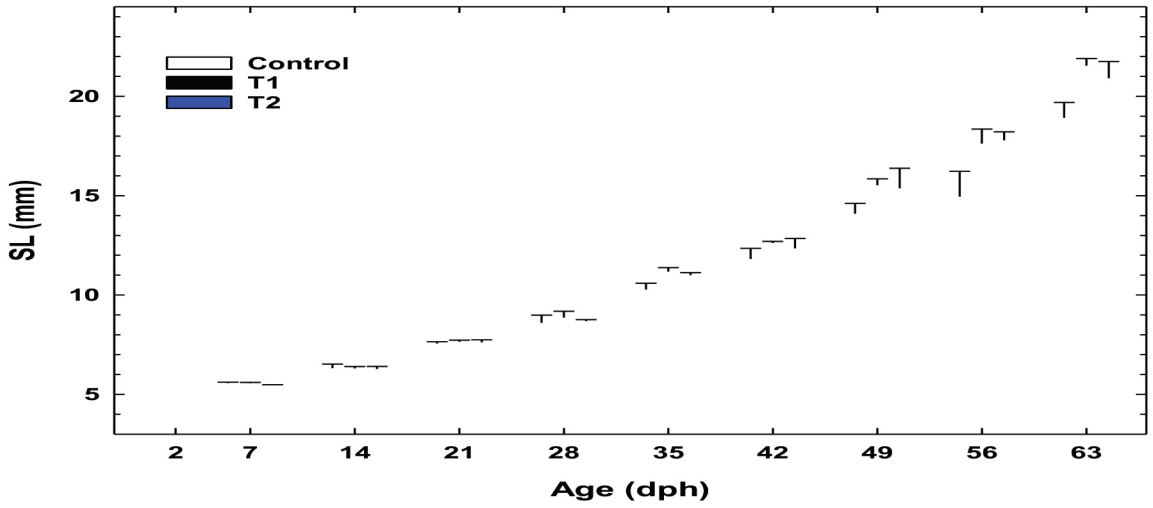
**Figure 1: Differences in standard length at different ages between treatments.**




**Results**

Growth rate, survival, and deformities were measured across treatments. Deformities in juveniles were analyzed using x-ray. Protein, fat, and mineral content in juveniles and live feed were also analyzed. The new enrichments (T1 and T2) resulted in increased growth rates compared to the control group, with no significant differences in deformities or survival between groups.

**Acknowledgements**

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# DEVELOPMENT OF AN EFFICACIOUS AND SAFE VACCINE AGAINST VIRAL NERVOUS NECROSIS (VNN) IN EUROPEAN SEA BASS *(Dicentrarchus labrax)*

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## Introduction

Viral Nervous Necrosis (VNN), also known as Viral Encephalopathy and Retinopathy (VER), is caused by a virus belonging to the genus Betanodavirus of the Nodaviridae family. The disease has been described in more than 50 fish species and is still considered a serious threat to aquaculture. Farmed European sea bass is severely affected, the virus is causing animal suffering and significant losses to the industry. Because of the need for preventive measures, Pharmaq developed a vaccine against VNN and the demand for vaccines is growing.

## Methods and Results

Development of the monovalent injection vaccine targeting Viral Nervous Necrosis Virus (VNNV) was initiated in 2012 and extensive research and documentation continued up to 2017 when the first Marketing Authorization was granted. Developing a robust and reproducible challenge model is key to be able to test and document vaccine efficacy. Protection was assessed clinically* through dose-response studies, evaluation of onset- and duration of immunity, and cross protection testing using challenge isolates of various origin. The safety of the vaccine, including local reactions such as abdominal adhesions and pigmentation, was investigated after vaccination. Extensive clinical testing under controlled laboratory conditions showed that the vaccine is safe to use and provides high levels of protection, which has been further supported by experience from the field. Data have also been gathered after large scale commercial use of the vaccine, and feedback from farmers has been vital to monitor efficacy and safety.

This presentation will review the highlights of the development process, with a special focus on clinical studies. Current field situation will also be addressed.

*The pre-clinical studies were conducted in line with the “License to Carry out Research on Animals” granted to AquaBioTech Group by the Ministry for Agriculture, Fisheries, Food and animal rights, Animal welfare Department in Malta and in accordance with procedures approved by the Zoetis Animal Ethics Committee.

# FROM SOIL TO SEA: OMEGA-3 BIOSYNTHESIS IN *Enchytraeus albidus* AND ITS POTENTIAL FOR AQUACULTURE

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**Introduction**

Methyl-end desaturases (or "ωx des") play a significant role in the biosynthesis of omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFA), biologically essential nutrients for vertebrates including fish. These enzymes are present in multiple aquatic invertebrates, including molluscs, cnidarians, crustaceans and annelids. Within the latter group is the terrestrial oligochaete *Enchytraeus albidus*, an organism often used as live feed in flatfish larviculture. While indirect evidence from lipid composition and transcriptomic analyses suggested that *E. albidus* is capable of synthesizing ω3 LC-PUFA, the responsible enzymes have not been characterized yet. This study aims to identify and characterize the repertoire of ωx des from *E. albidus*.

**Materials & Methods**

To identify the ωx des, tblastn searches were conducted against publicly available Transcriptome Shotgun Assembly (TSA) from *E. albidus*, using as a query known ωx des sequences from marine annelids. The putative ωx des sequences retrieved from *E. albidus* were used as a reference to design primers enabling the amplification of the complete open reading frames (ORF) by PCR. The PCR template was cDNA prepared from total RNA from fresh worms (pools of 3 ind). The PCR product corresponding to the ORF of the putative ωx des from *E. albidus* was digested and cloned into pYES2 for functional assays in yeast (see below). Correctness of the ORF inserts in pYES2 was confirmed by sequencing. For the molecular characterization of the ωx des, we compared the deduced protein sequences with other annelid desaturases, identified ωx des-like conserved domains, and performed a Maximum likelihood phylogenetic tree along with desaturase sequences from multiple invertebrates. For functional characterization, transgenic yeast transformed with the pYES2 constructs containing the *E. albidus* ωx des ORF, were used in two different assays. First, yeast expressing each ωx des gene were grown in the absence of exogenously added substrates, and their fatty acid profiles were then compared with those of yeast transformed with empty pYES2 (control) and grown under the same conditions (n=3). This assay informed about the ability of the *E. albidus* ωx des to desaturate yeast endogenous fatty acids. In a second assay, the transgenic yeast expressing each ωx des gene were supplemented with potential ω6 PUFA substrates (18:2ω6, 18:3ω6, 20:2ω6, 20:3ω6, 20:4ω6, 22:4ω6 and 22:5ω6). For both assays, yeast were harvested after 48h incubation, and total lipids were extracted for further derivatization into fatty acid methyl esters (FAME). FAME were analyzed through Gas Chromatography (GC-MS).

**Results**

We identified two distinct *E. albidus* ωx des, termed hereafter "ωx1" and "ωx2". Both *E. albidus* ωx des possess the three histidine boxes characteristic of methyl-end desaturases. The phylogenetic analysis successfully locates these proteins within one of the Annelida clades, and near other oligochaetes such as *Eisenia fetida* and *Glossoscolex paulistus* (Fig. 1). Despite their close phylogenetic relationship, the *E. albidus* ωx1 and ωx2 exhibited very different functions. The functional assays showed that yeast expressing ωx1, but not ωx2, were able to convert 18:1ω9 into 18:2ω6, confirming this enzyme is a Δ12 desaturase. However, the ωx2 showed Δ15, Δ17 and Δ19 activities, converting a variety of ω6 substrates into ω3 LC-PUFA such as EPA and DHA (Table 1)..

**Conclusions**

We have successfully identified and functionally characterized two ωx des from *E. albidus*. Both ωx des have conserved domains and are phylogenetically related. The functional assays demonstrated that ωx1 des is a Δ12 desaturase, whereas ωx2 can be categorized as an ω3 desaturase. While ongoing investigations are aiming at the characterization of front-end desaturases and fatty acid elongases with potential roles in the LC-PUFA biosynthesis in *E. albidus*, the present results confirm that this terrestrial annelid possess key enzymatic capacity required for *de novo* synthesis of LC-PUFA.

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**Table 1**. Substrate conversion of the ωx2 methyl-end desaturase from *E. albidus*. Results are presented as percentage (%) of the exogenously supplemented fatty acid (FA) substrates converted into the corresponding products.

| FA substrate | FA product | Conversion (%) | Activity |
|---|---|---|---|
| 18:2ω6 | 18:3ω3 | 16.13 | Δ15 |
| 18:3ω6 | 18:4ω3 | 48.89 | Δ15 |
| 20:2ω6 | 20:3ω3 | 20.52 | Δ17 |
| 20:3ω6 | 20:4ω3 | 19.24 | Δ17 |
| 20:4ω6 | 20:5ω3 | 18.29 | Δ17 |
| 22:4ω6 | 22:5ω3 | 2.02 | Δ19 |
| 22:5ω6 | 22:6ω3 | 11.93 | Δ19 |

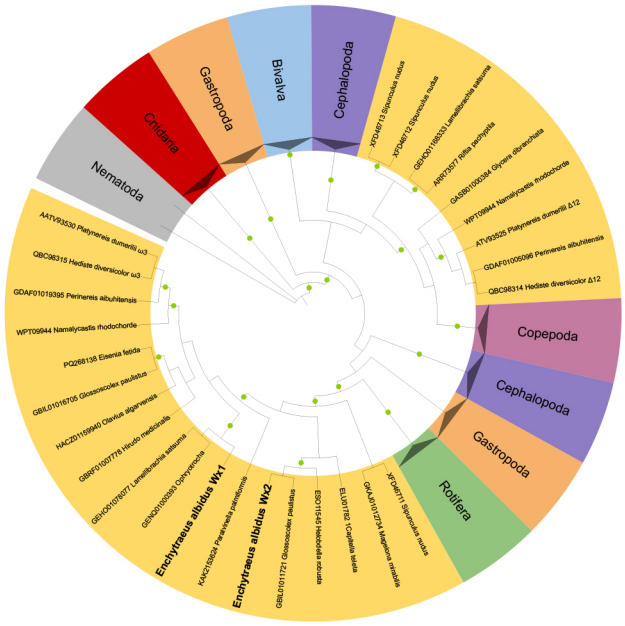


**Fig.1** Maximum likelihood phylogenetic tree of invertebrate ωx des. *E. albidus* ωx des are marked in bold. Bootstrap values (1000 iterations) with a support value of 90% or higher are highlighted in green. NCBI accession numbers are given for each sequence.

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# A RAPID RISK ASSESSMENT TOOL TO ENHANCE AND STANDARDISE AQUATIC ANIMAL HEALTH RISK MANAGEMENT UNDER FAST CHANGING AQUATIC ENVIRONMENTS

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## Introduction

Aquaculture has experienced significant growth since the 1980s, yet its expansion is increasingly constrained by disease outbreaks, which account for approximately 20% of production losses annually (Brummett and Hargreaves, 2019; Peeler et al., 2023). Risk assessments are integral to the development of adaptive and timely disease management strategies to counterbalance these losses, notably by evaluating disease risks, impact and mitigation measures (WOAH, 2024). Conducting risk analysis for aquatic animal health, however, presents several challenges, including the need for rapid response to sudden epidemiological events, limited knowledge of emerging novel threats, shifting ecological and socio-economic drivers and transparent communication of risks and uncertainties across diverse stakeholder groups. To address these, a Rapid Risk Assessment (RRA) tool was specifically developed to support strategic decision-making and effective aquatic animal health management, particularly under rapidly evolving environmental conditions.

## Materials and Methods

The RRA tool was developed using Microsoft® Excel® (Microsoft Corporation 2024) and is structured according to the World Organisation for Animal Health (WOAH) risk analysis framework (WOAH, 2024). The RRA begins with general assessment information that includes the pathogen name, a colour-coded risk summary, the assessor's name, versioning, completion date and scope. The second section of the RRA tool covers pathogen characterisation: pathogen description, disease pattern, host species (including their native status) and routes of disease introduction and spread. This informs the subsequent section of the RRA tool where standard terminology is used to provide qualitative likelihood estimates for each of the 3 core components of the risk assessment: introduction, establishment and consequence of establishment. The likelihood estimates are aggregated using risk matrices and result in qualitative risk estimates assigned with numerical scores. The RRA utilises internationally recognised terminology and standards for descriptors of likelihood and risk estimates, ensuring consistency and comparability across assessments (Horigan et al., 2023). The amount of uncertainty associated with each estimate is also incorporated to document gaps in knowledge (EFSA, 2018).

## Results

Rapid risk assessments are mainly conducted following the first occurrence of an event of public or animal health concern to facilitate the rapid collection of information and provision of advice to risk managers within a short period of time (de Vos et al., 2022). Yet, the RRA tool also provides a flexible framework to capture risk under future environmental conditions, specifically climate change. In this latter context, the RRA tool was applied to assess disease risks under current, 2°C (RCP4.5) and 4°C (RCP8.5) increased temperature scenarios (CCC, 2021). Rapid risk assessments were completed by reviewing the literature for minimum, maximum and optimal temperature ranges associated with disease viability, clinical expression and persistence of non-native host species under each climate scenario. The systematic, consistent approach provided a comprehensive ranking of diseases per risk and advice on prioritisation for future adaptation of aquatic animal health management.

## Discussion

The RRA tool has been developed for enhancing aquatic animal health management. It provides a structured, transparent and objective framework to rapidly support evidence-based decision-making and facilitate risk communication. As RRAs are completed, a library of RRAs is built which grows preparedness for emergency response and identifies knowledge gaps for further research and development.

This adaptable framework also facilitates direct comparison of existing, new or emerging aquatic disease threats in a systematic manner, allowing decision makers to plan disease management strategies under fast changing aquatic environments and ensure aquaculture resilience to future threats.

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# EVALUATING POULTRY BY-PRODUCT MEAL AND FISH OIL ALTERNATIVES ON GROWTH PERFORMANCE AND GUT HEALTH OF RAINBOW TROUT (*Oncorhynchus mykiss*): BENCHMARKING OF THE TRIAL RESULTS WITH FEEDNETICS™

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## Introduction

The increasing global demand for fish and seafood has led to significant growth in the aquafeed industry. However, this expansion has raised sustainability concerns due to the limited availability of marine-derived ingredients, such as fishmeal (FM) and fish oil (FO), which are essential in conventional aquafeeds. As the global production of these ingredients nears its maximum capacity, it is crucial to find sustainable alternatives to ensure the continued growth of the aquaculture sector. Over the past few decades, terrestrial plant-based ingredients such as soy, rapeseed, and maize have been used to partially replace FM and FO. While these ingredients show promise, they have limitations, such as an unbalanced amino acid and fatty acid profile, which can impact fish growth and health (Cardinaletti et. al., 2022). In recent years, protein alternatives from terrestrial animal by-products, such as poultry by-product meal (PBM), have gained attention as viable substitutes for FM (Psofakis et. al., 2020). PBM contains a balanced amino acid profile but may require supplementation with other protein sources like feather meal hydrolysate (FMH) to address deficiencies in lysine and methionine. In addition, alternative oils such as algae oil have been proposed as substitutes for FO, providing essential omega-3 fatty acids. This study aims to investigate the effects of a diet completely replacing FM with PBM and FMH, alongside the complete substitution of FO with algae oil and rapeseed oil, on the growth performance, gut health, and liver morphology of rainbow trout (*Oncorhynchus mykiss*). The results from the experiment were compared with the nutrient-based model FEEDNETICS™ (FEEDNETICS Software, SPAROS). The model was used to do a follow-up of the trial and assess whether the observed fish performance aligned with theoretical expectations.

## Materials and methods

Two experimental diets were formulated to evaluate the effects of poultry by-product meal (PBM) and fish oil (FO) replacement in rainbow trout (*Oncorhynchus mykiss*) diets. The control diet (FM) contained 15% fishmeal, 5% poultry meal (PM), and FO, while the PBM diet fully replaced FM with 15% PM and 5% feather meal hydrolysate (FMH). FO was substituted with 5.35% VeraMaris® algae oil and rapeseed oil. The diets were extruded at SPAROS facilities, Portugal. The 90-day feeding trial was conducted at "Fattoria del Pesce" in Cerano, Italy. Juvenile rainbow trout (n = 2,908; initial weight = 27.5 ± 0.3 g) were randomly distributed in four 10 m³ outdoor tanks (727 fish/tank, initial density: 20 kg/m³). Following a one-week acclimation, fish were fed once daily at 1.5–2.0% biomass. Water quality parameters were monitored regularly. Growth performance was assessed by monthly weighing, and daily mortality was recorded. The data were entered into FEEDNETICS™ to model fish growth based on dietary inputs.

At the end of the trial, 32 fish per diet were sampled for histological evaluation (liver and intestinal morphology), short-chain fatty acid (SCFA) analysis, and fillet fatty acid composition. Histological samples were stained with hematoxylin-eosin and analyzed using microscopy. SCFAs were quantified using HPLC, while fatty acid profiles were determined via gas chromatography. Statistical analyses were conducted at the individual fish level using Student's t-test or Mann-Whitney test ($p < 0.05$).

## Results and Discussion

### Growth Performance and Feed Utilization

Rainbow trout readily accepted both diets, and the 90-day feeding trial showed no significant differences in survival rates (>97%), final body weight (FM: 170.80 ± 7.61 g, PBM: 166.91 ± 6.62 g), or feed conversion ratio (FCR: FM = 1.33 ± 0.05, PBM = 1.35 ± 0.04) between dietary groups. The specific growth rate (SGR) was similar ($p > 0.05$) between fish fed FM (2.03 ± 0.04 % day$^{-1}$) and PBM (2.00 ± 0.08 % day$^{-1}$). No external or internal lesions were observed in either group.

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**Intestinal and Liver Histomorphology**

Histological examination revealed well-organized intestinal tissue in both groups, with significantly longer intestinal folds ($p < 0.05$) in the anterior intestine of PBM-fed fish. However, the PBM group exhibited a significant increase in submucosa thickness in the posterior intestine ($p < 0.05$), possibly indicating a mild inflammatory response. Liver histology showed normal parenchymal structure in both feeding groups, with PBM-fed fish exhibiting reduced hepatic lipid accumulation compared to FM-fed fish.

**Fecal Short-Chain Fatty Acids (SCFAs) and Fillet Fatty Acid Composition**

SCFA analysis indicated significantly higher acetate (18.73 mmol/L) and butyrate (0.91 mmol/L) levels in PBM-fed fish. Fatty acid composition analysis revealed lower saturated fatty acids (SFAs) and significantly higher polyunsaturated fatty acids (PUFAs), including EPA+DHA, in PBM-fed fish, with improved nutritional indices (atherogenic and thrombogenic indices).

**Benchmarking of the trial results with FEEDNETICS ™**

The FEEDNETICS™ application model, served as a reference point and supported the conclusion of this study, in terms of the performance of fish fed diets with PBM and natural algal oil.

**Conclusions**

Replacing 20% of fishmeal with PBM and FMH, along with fully substituting FO with VeraMaris® algae oil, sustained growth performance, gut morphology, and liver health in rainbow trout. Although fillet PUFA levels decreased, improved nutritional indices suggest potential human health benefits. Additionally, the FEEDNETICS™ model provided valuable validation, reinforcing the observed performance of fish fed PBM- and algal oil-based diets. These findings underscore the potential of PBM and algal oil as sustainable, nutritionally balanced alternatives for environmentally friendly trout diets.

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# COMPARATIVE IRON REGULATED PROTEIN EXPRESSION IN THE FISH PATHOGEN *Aeromonas veronii* UNDER IRON STRESS

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**Introduction:**
*Aeromonas veronii* is a bacterial pathogen infecting both freshwater and marine aquaculture species. *A. veronii* infection results in *hemorrhagic septicemia* and epizootic ulcerative syndrome leading to severe pathology, mortalities and economic losses particularly in European seabass (*Dicentrarchus labrax*) and Nile tilapia (*Oreochromis niloticus*). Like other Gram-negative bacteria, *A. veronii* the outer membrane (OM) is essential for bacterial survival, virulence, and adaptation, particularly under iron-limited conditions. Since iron availability is restricted in host environments, *A. veronii* has evolved specialised iron uptake systems and iron-regulated outer membrane proteins (OMPs) to enhance survival. Understanding these adaptations is crucial for developing targeted antimicrobial strategies and identifying protective antigens. This study compares the OMP expression profiles of three commercially relevant *A. veronii* isolates grown under iron limitation and standard laboratory conditions, aiming to identify OMPs associated with the adaptation to iron-limited environments.

**Method:**
Three *A. veronii* strains (NK01 (tilapia), NS and PDB (seabass)) were cultured in TSB medium with/without 100 μM 2,2'-dipyridyl. OMP fractions were obtained using a sarcosine-insoluble isolation method (Huang et al., 2006). Proteins were digested with trypsin using single-pot solid-phase enhanced sample preparation (SP3) and analysed by Orbitrap Exploris mass spectrometry in data-independent mode (DIA). Differential expression of OMPs was analysed using the R package prolfqua (Witold et al., 2023)

**Result:**
Iron limitation resulted in significant changes in the OM proteome of *Aeromonas veronii* strains NK01, NS, and PDB. OMPs associated with iron uptake, membrane transport, and virulence were upregulated in all three strains including TonB-dependent siderophore receptors (CF123_19915 and CF123_11360), a lipocalin (CF123_10435), a ligand-gated channel protein (CF123_06935), the Type IV pilus biogenesis protein PilW, and several autotransporters and general transporters (Figure 1). In addition, proteins of key iron acquisition systems, including ABC transporters, ferrous iron transporters, and heme utilisation proteins were significantly upregulated. These results suggest a coordinated adaptation to iron stress particularly the upregulation of TonB-dependent siderophore receptors which play a crucial role in transporting iron-chelating compounds from the outer membrane to the periplasm, enabling the bacterium to extract iron from the environment and contributing to iron homeostasis (Wang et al., 2017). In contrast, several OMPs involved in export or non-essential transport, such as the TonB-dependent copper receptor (CF123_17620), PilQ (CF123_07595), the Type II secretion system protein GspD, and porins, (OmpA, LamB, OprD family porin, OmpW, maltoporin and OmpA (CF123_10155)) were significantly downregulated. Components of the electron transport chain and other iron-dependent metabolic enzymes were also downregulated. Our findings are consistent with previous data, which suggests that under iron-limited conditions, bacteria suppress the expression of OMPs associated with non-critical permeability or export functions (Zhang et al., 2020). This dual response indicates a strategic metabolic adaptation to conserve energy and enhance iron uptake under iron-limited conditions.

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**Conclusion:**

Our findings show that *A. veronii* undergoes a co-ordinated regulatory response under iron limiting conditions. We observed a significant increase in key iron acquisition proteins, including TonB-dependent siderophore receptors, ABC transporters, ferrous iron transporters, and heme utilisation proteins, which enhance iron transport and maintain cellular iron homeostasis. Conversely, expression of non-essential OMPs in the response to iron limitation such as components of the electron transport chain, iron-dependent metabolic enzymes, and several other OMPs were significantly downregulated to alleviate the stress caused by iron limitation. Overall, this mechanism is vital for the adaptive regulation of *A. veronii* under metal ion-limited conditions. A comprehensive understanding of how bacteria acquire iron in metal ion-deficient conditions supports the invasion mechanism of *A. veronii* disease.

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# AQUASTAR: AUTONOMOUS UNDERWATER SYSTEM FOR TARGETED ASSESSMENT & REPAIR

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## Introduction

The AQUASTAR project targets combined autonomous inspection and repair of holes in sea-based aquaculture net pens. Holes in the net represent the main reason for fish escape and is a challenge in aquaculture fish farming in traditional net pens.

## Motivation

Net inspections are currently carried out manually and include a remotely operated vehicle (ROV) and an ROV pilot. However, inspecting net patterns for hours on end is tedious and can increase the risk of overlooking holes. Divers are usually employed for repair; however, they are exposed to risks that may lead to injuries and even death. This strongly advocates for both enhanced hole detection systems and alternative net repair solutions.

The AQUASTAR project will develop solutions for ROVs with manipulators enabling automatic hole localization, automatic vehicle positioning and hole repair. This will serve as a tool for ROV pilots to achieve enhanced net inspections and the opportunity to immediately repair holes in the net.

## Project status and project results

AQUASTR has developed and tested a net-relative navigation system that allows for horizontal and vertical movement and station keeping relative to the net. The project has also developed a fully operational automatic hole detection system, which, to the best of the project consortium's knowledge, marks the first time hole detection is being used commercially. There is an on-going process of gathering images of holes in the net that will be published as open-source datasets. The datasets will include several varying conditions present in aquaculture, (e.g., different illumination, turbidity and biofouling conditions. Moving further, the project aims to develop hole-relative station keeping and control strategies for combined vehicle-manipulator systems for hole repair.

## Acknowledgments

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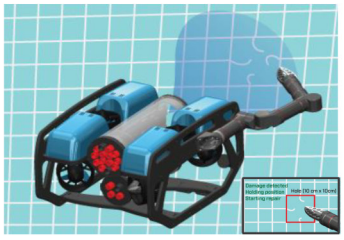

NJORD AQUA Måsøval SINTEF NTNU Forskningsrådet

# PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) CULTURED IN RECIRCULATING AQUACULTURE SYSTEMS (RAS). EFFECTS OF DENSITY ON GROWTH, MORTALITY, AND WELFARE

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**Background**
Aquaculture is a rapidly growing industry and one of the most vital pillars in addressing the increasing global demand for food. Different farming systems and strategies have been developed to achieve more modern and innovative ways of farming, involving automated and more accurate technologies, such as recirculating aquaculture systems (RAS). The Pacific white shrimp, *Litopenaeus vannamei*, is one of the most common species in shrimp farming. *L. vannamei* are usually cultured in industrial monoculture systems, hence, few studies have investigated the effect of using RAS technology to cultivate *L. vannamei*. This study aimed to investigate the impact of two stocking densities (Low Stocking Density, LSD, initial density 165 individuals/m2 and High Stocking Density, HSD, initial density 264 individuals/m2) on the growth, mortality, and welfare of *L. vannamei* cultured in a clear-water RAS.

**Experimental setup & data collection**
There were two independent clear-water RAS, and each system had two tanks. The experiment had four tanks in total. The two tanks connected to system 1 had a stocking density of 165 individuals/m2 (defined here as low density, LSD). The two tanks connected to system 2 had a stocking density of 264 individuals/m2 (defined here as high density, HSD). The mean weight was 0.35 g when the grow-out phase was initiated. Water quality parameters such as temperature, salinity, oxygen, and pH were measured daily. Water quality analysis was performed weekly to monitor ammonia, nitrite, and nitrate concentrations. Length, weight and welfare parameters were measured every fifteen days throughout the experiment. For each sampling, 10 shrimp was retrieved from each tank to be inspected and measured.

**Results**
The overall mean salinity was significantly different between the two treatments. The salinity fluctuated in both treatments, but the HSD experienced more frequent fluctuations than the LSD. The oxygen saturation fluctuated throughout the experiment and was significantly different between the LSD and HSD systems. The HSD system was the only system that experienced an oxygen saturation level below the optimal range. There was no significant difference in the specific growth rate (SGR) between treatments. The LSD and HSD systems had an overall SGR of 4.84 %/day and 4.71 %/day. During the last sampling, the mean weight varied significantly between treatments, where the mean weight was significantly higher for the LSD system. The mean length showed a tendency towards differences at the two final sampling points. Mortalities were observed throughout the experiment. An incident of high mortality (5 % of total biomass was lost) occurred on day 1 of the experiment following handling and distribution at start-up. Approximately 21–28 days into the grow-out phase the HSD system experienced a brief period of high mortality (8.6 % of total biomass was lost in one week) due to a disease called white muscle necrosis. The overall welfare of the shrimp was estimated by combining the results from indirect welfare indicators, individual welfare indicators, and daily observations that were made. There was a significant difference in animal welfare between treatments of LSD and HSD. Specifically, there was a significant difference between antennae damage between the treatments, where HSD scored significantly lower than LSD. There was no significant difference for the other individual welfare indicators. Overall, there was a significant difference in survival between treatments. There was a significant difference in animal welfare between treatments of LSD and HSD.

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**Conclusions**

The overall growth of *L. vannamei* was satisfactory although similar between treatments. Survival was poor for both treatments, but significantly lower in the HSD group, most likely due to the occurrence of muscle necrosis and unforeseen events that caused mortality. The welfare assessment technique was sufficient, but a significantly poorer welfare in the HSD system was observed. This study found that stocking density do affect the mortality and welfare, but not growth of *L. vannamei* cultured in RAS.

**Future research**

It has been well documented for biofloc systems and ponds that density does affect the growth of *L. vannamei*, and in the current study a significant difference in mean weight was observed during the last sampling. It is therefore suggested that more research on the cultivation of *L. vannamei* in RAS is needed.

# ACUTE EFFECTS OF INORGANIC MERCURY ON RAINBOW TROUT (*Oncorhynchus mykiss*) HEART EXCITABILITY

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## Introduction

Mercury contamination in aquatic environments poses a potential risk to aquaculture species, as it can impair fish health, growth, and survival. Cardiotoxic effects of mercury may compromise performance and stress tolerance in farmed fish, especially under intensive rearing conditions. Mercury ($Hg^{2+}$) is a potent environmental toxin and a persistent environmental problem, with inorganic mercury ($Hg^{2+}$) being a significant pollutant in aquatic ecosystems. Fish cardiovascular physiology is particularly sensitive to chemical stressors, and disturbances in heart excitability can impair survival. While organic mercury forms have been widely studied, the acute cardiotoxicity of inorganic mercury in fish remains less understood. This study aimed to investigate the acute effects of $HgCl_2$ on rainbow trout (*Oncorhynchus mykiss*) heart excitability at whole-heart, cellular, and molecular levels. We hypothesized that $Hg^{2+}$ disrupts cardiac ion channel function, leading to arrhythmias and altered electrophysiological parameters.

## Methods

We used ex vivo perfused hearts, isolated ventricular myocytes, and patch-clamp techniques to assess responses to 0.1–10 μM $HgCl_2$. ECGs were recorded to measure heart rate, PR, QRS, and QTc intervals, as well as T-wave amplitude. Action potentials were recorded in current-clamp mode, analysing $APD_{50}$, $APD_{90}$, and $APD_{10}$, resting membrane potential, and upstroke/repolarization rates. Voltage-clamp experiments quantified effects on $I_{Na}$, $I_{CaL}$, $I_{Kr}$, and $I_{K1}$. Statistical analyses included ANOVA and Hill equation fitting for $IC_{50}$ values.

## Results

$Hg^{2+}$ induced concentration-dependent arrhythmias, including bradycardia (50–57% HR reduction at 6–10 μM), atrioventricular block, and QTc prolongation. Action potentials showed prolonged $APD_{50}$ and $APD_{90}$, shortened $APD_{10}$, and early and delayed afterdepolarizations. $I_{Na}$ ($IC_{50}$: 0.2 μM), $I_{CaL}$ ($IC_{50}$: 0.7 μM), and $I_{Kr}$ ($IC_{50}$: 1.0 μM) were dose-dependently inhibited, whereas $I_{K1}$ was unaffected.

## Conclusions

Inorganic mercury is acutely cardiotoxic to rainbow trout, disrupting ion channels and causing arrhythmias that may impair cardiac output. These findings highlight the ecotoxicological risk of $Hg^{2+}$ in fish, particularly in polluted aquatic environments, with implications for aquaculture operations facing multiple stressors, such as elevated temperature and intensive farming conditions.

# DIFFERENTIAL IMPACT OF TWO JELLYFISH SPECIES ON RTGILL-W1 CELLS

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## Introduction

Gills are a multifunctional organ which is responsible for gas-exchange, ion-regulation and osmoregulation, amongst others. It is therefore of upmost importance for fish well being and health to have functional gills. Unlike lungs of terrestrial animals, gills are constantly exposed to the environment and as a result more prone to adverse effects. It is well established that jellyfish can damage – and cause mortalities in fish. Fish can either die as a direct result from the jellyfish venom, or as a result of secondary infections. Jellyfish can also damage the gills, which will negatively affect the overall health of the fish.

## Materials and methods

In vitro studies were carried out to determine the effects of two types of jellyfish, *Aurelia aurita* and *Apolemia uvaria*, on a rainbow trout gill cell line (RTgill W1, from ATCC). In order to obtain the optimal concentration and exposure time of macerated jellyfish, we performed a dose response study. We utilized measurements of cell proliferation, ATP and cytotoxity in order to determine which exposure time and concentration to use in further experiments.

After finding the optimal concentration and exposure time, we performed a pre-exposure to a compound stressor experiment where we first exposed the cells to either $H_20_2$ or LPS before exposing them to *A. aurita* and *A. uvaria*. Thereby we examined genes of interest by utilizing qPCR.

## Results

We discovered that after the longest exposure time and the highest concentration of *A. uvaria*, almost all of the RTgill W1 cells had died. We found that after 24 hours of exposure to *A. aurita* and *A. uvaria* proliferation decreased in a concentration dependent matter. Regarding ATP-levels, we observed an increase in cells exposed to *A. uvaria* for 4 hours, and a decrease in cells subjected to *A. aurita*. For cells exposed to jellyfish for 24 hours, there was a concentration dependent decrease. We observed an increase in cytotoxicity after both 4 and 24 hours of jellyfish exposure with increasing concentrations.

Regarding the experiment with pre-exposure to a compound stressor we discovered different results on apoptosis related genes. For instance, in cells treated with *A. uvaria* had an increase in caspase 8, caspase 7 and caspase 3a2 compared to the control group. For CK11, a gene involved in immune regulation, we also observed an increase in cells treated with *A. uvaria* compared to the control group.

## Conclusion

We discovered that the optimal concentration and exposure time, that achieved a response in the cells but still had good survival, was 2 % macerated jellyfish for 4 hours. Furthermore, we discovered that *A. uvaria* increases the relative expression of apoptosis related genes and an immune regulatory gene. These results indicate that A. uvaria damages the cells in such a way that the cells induces apoptosis. This suggests that A. *uvaria* has the ability to harm the gills directly, not only because of secondary infections.

# FROM FIELDS TO FJORDS; A FRAMEWORK FOR ENHANCING FISH PERFORMANCE AND RESILIENCE ACROSS GLOBAL AQUACULTURE ENVIRONMENTS

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## Introduction

Selective breeding in Atlantic salmon (*Salmo salar*) has delivered substantial gains in productivity, disease resistance and other key traits. However, these gains are often not realised consistently across the heterogeneous environments in which salmon are farmed worldwide. This inconsistency is explained by genotype-byenvironment (GxE) interaction, where genotype performance and rankings can change between environments, reducing selection accuracy and slowing genetic progress (Gonzalez et al., 2022; Sae-Lim et al., 2016; Tollervey et al., 2024).

In aquaculture, GxE interaction is particularly pronounced due to the high sensitivity of fish to stressors, such as temperature, parasites, and handling (Sae-Lim et al., 2014). Environmental variability, both within and between locations (Fig.1), reduces resilience of Atlantic salmon for complex traits like growth and stress tolerance. This generally manifests as unpredictable shifts in performance across environments, meaning that genotypes bred for "optimal" conditions may underperform in commercial production systems subject to environmental challenges.

## Objectives

This project introduces a novel framework for integrating GxE interaction, into aquaculture breeding strategies, to improve environmental resilience across global aquaculture environments, which is being accelerated by climate change.

The specific aims are to (1) quantify changes in genetic gain and environmental resilience over time, (2) assess the extent of genotype reranking across production en ironments, and (3) identify index weights that optimise performance and resilience.

## Methods

Using AlphaSimR (Gaynor et al. 2021), genotypes for 5 founder populations of Atlantic salmon were simulated and mated randomly, subsequently being selected for growth to create a 'domesticated' base population. Environmental structure was then introduced using real-world climate data from four global regions: Scotland, Norway, Tasmania, and Chile to reflect realistic variation in production environments. These environments differ greatly in their timing, frequency and magnitude of heat stress events (Fig. 1).

The simulation had three key traits: *Baseline growth* - performance under optimal conditions, *Environmental sensitivity* - performance under stress conditions, *Stress recovery* - performance following exposure to stress. These traits were used to (i) calculate novel 'resilience' traits as deviations from normal growth when under stress, (ii) construct weighted selection indices that combine productivity and resilience, and (iii) quantify the value added of including resilience into breeding objectives.

## Results

The three growth traits were initially explored in a model environment with equal time under optimal, stress and recovery conditions (Fig. 2) to assess the decay of the correlation between expected growth and realised growth due to the stress (Fig. 3). The parameters of the stresses experienced were varied to show the change in decay when the start and duration of stress is unpredictable (Fig. 4).

The simulations in this project demonstrate how selecting for resilience traits, alongside traditional performance metrics, can improve the stability of genotype performance across diverse environmental conditions. The framework can be used to balance selecting for performance in "optimal" environments and selecting for resilience in variable ones. This trade off can be navigated by incorporating informed selection indices to find the optimal balance of productivity and environmental resilience, helping identify and breed for genotypes with more predictable and stable performance in the face of a changing environment.

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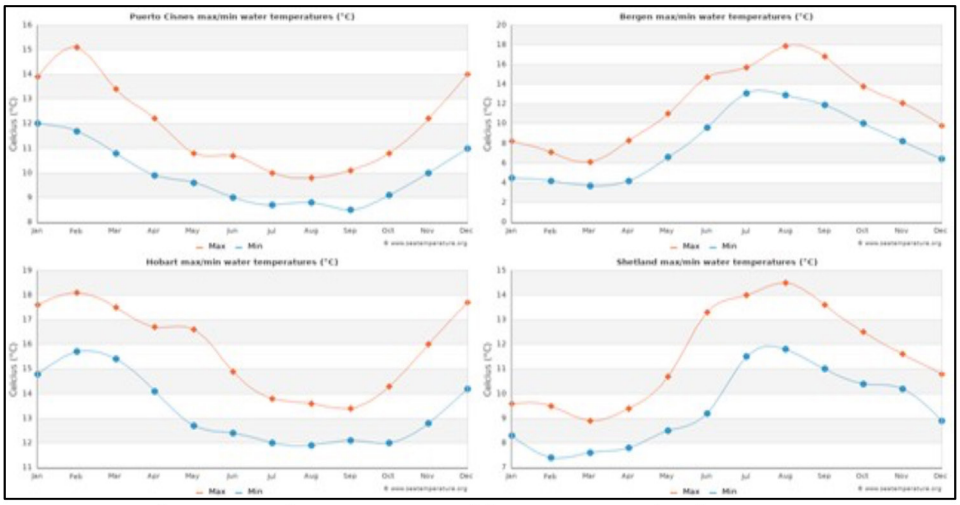


(2) assess the extent of genotype reranking across production

*Figure 1: Average monthly sea surface temperatures for key salmon production sites in (top left to bottom right) Chile, Norway, Tasmania and Scotland. The blue line represents the minimum temperature and orange line is maximum temperature. Source: https://www.seatemperature.org/*

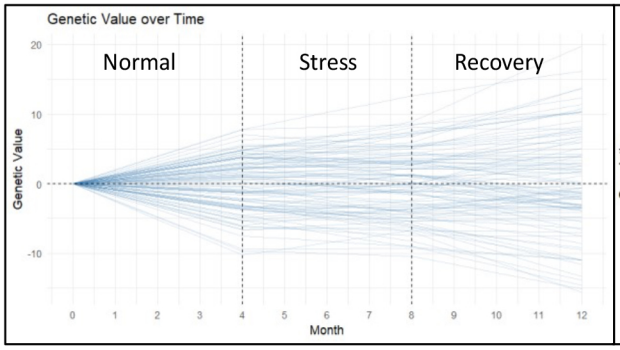


*Figure 2: Growth for 100 genotypes as deviations from the population mean, as the environment changes from optimal realised conditions (0-4 months), under stress (4-8) and recovery (8-12).*

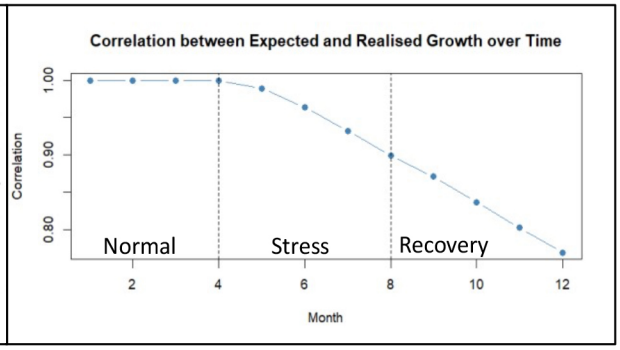


*Figure 3: Plot showing the decay of the corelation between expected growth rate under optimal conditions and the growth rate due to stress.*

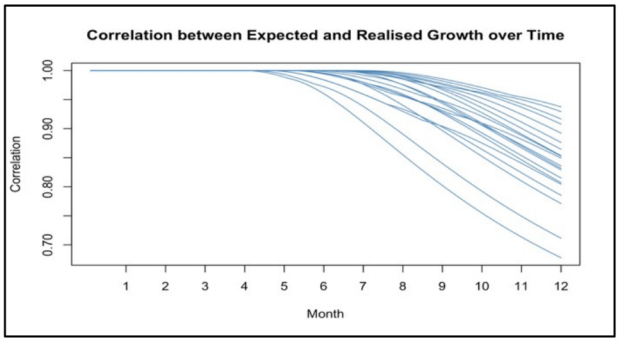


*Figure 4: Plot showing the decay of the corelation between expected growth rate under optimal conditions and the realised*

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## Conclusions

The new framework enables aquaculture breeding programmes to effectively leverage GxE interaction, contributing to the development of salmon stocks that are resilient to environmental change. This framework can be extended to numerous other commercially important traits and production systems, to improve long-term productivity and sustainability under the emerging pressures of climate change.

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# BUSINESS MODELS FOR EUROPEAN ALGAE ENTREPRENEURS; A REVIEW OF FINDINGS FROM THE ALGAEPROBANOS PROJECT

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## Introduction

AlgaeProBANOS is a Horizon Europe project that aims innovation and market access pipelines for accelerating 6 highly relevant algae value chains, namely food, feed, textiles, nutraceuticals, biostimulants and cosmetics, covering both microalgae (two value chains) and seaweeds (macroalgae, four value chains). To help the products developed within the project reaching the global market (see Selnes et al., 2021), sustainable and circular business models are developed and financial analyses of the algae products are conducted. These business models help the sector identify its strengths, and relevant upgrading strategies (van den Burg et al., 2021). This contributes to communicating the commercial value of algae products and companies to potential investors and stakeholders. The economic insights from these six value chains can pave the way for future developments in the wider algae sector.

## Materials and methods

The project uses a collaborative co-creation process to identify the business models currently in place in the European algae sector, using the Business Model Canvas as framework. The companies are actively involved in developing and discussing the business model, with the research team analysing the information provided. Business models will be informed by strategic financial analysis which will quantify the revenue generating potential, capex and operating costs, the margins and operating profit, and payback period and break-even volumes of proposals. Additional insights about developments in the business (e.g. changes in technical processes or partners, trading market and the motivation behind them) are collected throughout the project. The project then zooms out to the wider European algae sector by incorporating lessons learned via a survey and interviews with other companies, which are external to the project.

## Results

The first results indicate that, contrary to observations made a few years ago (Selnes et al., 2021), many companies are moving away from cultivating seaweed. Compared to harvested from the wild, cultivated seaweed can currently not compete in costs. For companies in the macroalgae business, the focus is often on the processing of wild harvest. However, another barrier is that European prices cannot compete with Asian prices. This means that growing a business based on wild harvested macroalgae is challenging as well. Instead, companies focus on the end of the value chain and try to increase the value of their products by using seaweed extracts and processing it into high-value products for the nutraceutical and cosmetic market. This switch in the focus of companies aligns with the observation that algae as food gets less attention in the debate. Obstacles like the Novel Food regulation, a lack of certification and standards, concerns about iodine and heavy metal levels, and low consumer acceptance have stagnated the sector. There is a reliance on few and bigger companies now to make a success of new algae products in different value chains.

## Discussion & Conclusion

The European algae sector is in development and strong support from the EU and national governments pushes development and innovation. Numerous business venture into the processing and development of products from seaweed, supported by investors. The review of business models however also shows the difficulties that companies experience to develop commercially viable products. Recognizing the potential of the algae sector to contribute to the provision of sustainable natural resources from Europe, it is pivotal to further support development of the sector. Future support is also justified given the positive impacts of seaweed cultivation and use, for nature restoration, climate mitigation and adaptation.

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# THE SINGLE-CELL ATLAS OF EARLY DEVELOPMENT IN ATLANTIC SALMON

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As they develop in eggs exposed to the environment, fishes are especially susceptible to external influences during embryogenesis. These include temperature and light, which have been shown to have long-term fitness effects. In the SALMOCODE project, we use single-cell transcriptomics to investigate these vulnerabilities in the Atlantic salmon embryo.

In the first phase of this project, we have sampled eggs of farmed salmon between 124 and 238 day-degrees of development, corresponding to the interval between early organogenesis and the 'eyed' stage. By sampling every day, we have created a time lapse consisting of 23 developmental stages, which we have used for single-cell library preparation using the Parse Biosciences Evercode WT Mega kit. After sequencing, the current atlas contains data on 50000 cells, which belong to the approximately 50 broad cell types that emerge during early development.

In our main time lapse experiment, conducted at 4 °C, survival was high; however, in concurrent experiments at elevated temperatures, mortality was very high, and concentrated around specific developmental time points. We are currently profiling survivors of these bottlenecks using single-cell transcriptomics, under the hypothesis that they might show temperature-induced deviations from healthy organ development. These deviations could, in turn, have a negative impact on resilience later in life.

For the second phase of the project, we have sampled eggs more broadly between gastrulation and hatching, and under both controlled (4 °C, no light, commercial domesticated genetic background) and natural conditions (changing environmental conditions during the Norwegian winter, wild Atlantic salmon). After processing using single-cell transcriptomics, these will be combined with the phase one atlas to form a comprehensive, reuseable reference for early development.

# IMPACT OF NOVEL FEEDING PROTOCOLS ON ORGAN DEVELOPMENT AND SKELETAL ANOMALIES IN ATLANTIC COD LARVAE (*Gadus morhua*)

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## Introduction

Aquaculture of Atlantic cod (*Gadus morhua*) continues to face some bottlenecks, particularly during larval rearing. Optimizing rearing protocols and customizing nutrition is essential not only for promoting larval growth and survival, but also for supporting organ ontogeny and skeletogenesis during the critical early life stages. During early ontogeny, the digestive and musculoskeletal systems undergo rapid morphological changes, and tailored nutrients play a critical role in supporting maturation, enhancing digestive capacity, and promoting proper skeletal formation. Although enriched rotifers and Artemia remain the benchmark live feeds in cod larviculture, inclusion of natural zooplankton such as barnacle nauplii has yielded improved growth, survival, and larval quality in several species, particularly in cod larvae (Øie et al., 2017). Despite comprehensive research into cod larval development and feeding protocols, knowledge on species-specific nutritional needs remains scarce. Given that the composition of weaning microdiets can influence appetite, gut development and bone formation, there is a pressing need to evaluate customized feeding regimes that combine novel live-feeds with tailored microdiet formulations. Therefore, the present study tested feeding protocols incorporating plankton eggs (Cryo-µ) and barnacle nauplii (Cryo-S, Cryo-L), alongside with customized microdiets, to assess effects on growth performance, skeletal anomalies, and organ ontogeny in Atlantic cod larvae.

## Material and methods

Two independent trials were conducted to evaluate the effects of different feeding protocols and novel microdiets on the growth performance, incidence of skeletal anomalies, and organ ontogeny of Atlantic cod (*Gadus morhua*) larvae. In both trials, larvae were obtained from ODE AS (Norway) and randomly distributed at 2 days after-hatch (DAH) into triplicate 400 L tanks. Rearing conditions were identical in both trials, with 24h light and water temperatures ranging from 8.8 to 10.8 ± 0.2°C. Trial 1 was conducted from 3 to 66 DAH and consisted of three treatments: CONTROL, D1, and D2. The CONTROL group was fed rotifers, large barnacle nauplii (Cryo-L), and a commercial microdiet. Treatments D1 and D2 received a diversified live feed protocol composed of rotifers, plankton eggs (Cryo-µ), small barnacle nauplii (Cryo-S), Cryo-L, in addition to experimental microdiets Diet 1 and Diet 2, respectively. Trial two, lasting from 3 to 67 DAH, also included three treatments: CONTROL, D3, and D4. In this trial, all groups were fed the same live feed protocol (rotifers, Cryo-µ, Cryo-S, and Cryo-L). The CONTROL group received the commercial microdiet, while D3 and D4 were fed experimental microdiets Diet 3 and Diet 4, respectively. Sampling was conducted at day 3, 30, 50, and at the end of the trial (66 and 67 DAH). Growth parameters, skeletal anomalies and organ development were assessed.

## Results

Experimental groups D1 and D2 showed earlier signs of gill development at 15 DAH and improved ontogeny of gut, liver and eye at 30 DAH, when compared to the control group (Figure 1A). At the end of the trial, both experimental groups showed lower incidence of skeletal anomalies. Particularly D1 (Figure 1B) and D4 (trial 2, results not shown) presented lower incidence of skeletal anomalies ($P<0.05$) than the control group (SK).

*(Continued on next page)*

A

| 15 DAH | D1 | D2 | CTRL |
|---|---|---|---|
| Anterior Intestine | 3 | 2 | 2 |
| Posterior Intestine | 4 | 3 | 4 |
| Liver | 2 | 2 | 2 |
| Eyes | 3 | 3 | 3 |
| Gills | 3 | 3 | 1 |
| **30 DAH** | **D1** | **D2** | **CTRL** |
| Anterior Intestine | 4 | 3 | 4 |
| Posterior Intestine | 4 | 4 | 2 |
| Liver | 4 | 4 | 2 |
| Eyes | 4 | 3 | 3 |
| Gills | 3 | 2 | 2 |

B

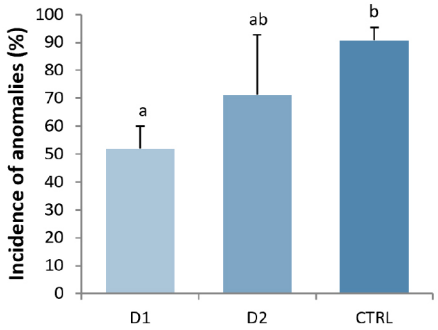


**Figure 1.** (A) Histology score for each organ of cod larvae at 15 DAH and at 30 DAH, according to the scoring system of Pacoring et al, 2022. The scale is from 1 (very poor organ development) to 5 (very good organ development). (B) Incidence of skeletal anomalies in Atlantic cod (66 DAH) fed two experimental (D1 and D2) and one commercial (SK) microdiets, in trial 1. Results are expressed as means + standard deviation. Different lowercase letters indicate significant differences between the dietary treatments ($P<0.05$).

## Discussion and Conclusion

The impact of the feeding regime on growth, organ ontogeny and skeletal anomalies of Atlantic cod larvae was evaluated in the present study. In both trials, there was no relevant effect of the experimental feeding regimes on growth performance, with RGR ranging from 10-10.7% (results now shown) and standard length within the same range or above values previously reported in studies using live feed protocols supplemented with natural zooplankton (Folkvord et al., 2017). Nevertheless, in trial one, experimental groups D1 and D2 showed earlier signs of organ development at 15 and 30 DAH when compared to the control group (Fig.1A), indicating that that the live feeding regime combining Cryo-µ, Cryo-S and Cryo-L has a positive effect on organ ontogeny, potentiating earlier cell development and differentiation. Such effect was also observed in trial two – cod larvae organs showed early development across all treatments at 35 DAH (results not shown). Remarkably, from 46-67 DAH cod larvae fed Diet 4 experienced a higher degree of cell differentiation when compared to the control group, hence demonstrating the potential synergistic effects of early feeding protocols with Cryoplankton and customized microdiets. Regarding skeletal development, both trials showed that experimental groups had a significantly lower incidence of skeletal anomalies (Fig. 1B), including fewer severe deformities (data not shown). This strongly suggests that the nature of Phospholipid (PL) sources in the experimental diets promote proper bone formation and larval tissue maturation. In marine fish larvae, dietary phospholipids have been shown to enhance not only vertebral ossification and reduce deformities but also support digestive enzyme activity and histological development. In Atlantic cod specifically, limitations in endogenous PL synthesis during early development mean that dietary PL is critical for both lipid transport and membrane formation, indirectly influencing organ differentiation and ontogenetic maturation of the intestine and liver. Overall, these findings support the hypothesis that the enhanced skeletal and organ development observed in the experimental treatments is closely associated with the synergistic effects of combining cryoplankton with tailored microdiets. In particular, the nature of phospholipid sources included in the microdiets appears to play a pivotal role in promoting the physiological maturation and skeletal development of cod larvae, ultimately contributing to improve juvenile quality.

## Acknowledgements

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# AQUACULTURE BY-PRODUCTS: A SOLUTION TO REDUCE THE USE OF FISHMEAL IN A CARNIVOROUS FISH PRODUCTION

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Over 35% of global fishery stocks are overexploited (FAO, 2022), partly driven by the demand for small pelagic fish to feed carnivorous farmed species. To address this, the aquaculture sector has significantly reduced fishmeal (FM) in feeds, from 50% in 1997 to 14% in 2017 (Naylor et al., 2021). Yet, overall FM use continues to rise due to rapid aquaculture growth. While plant proteins like soy have helped lower FM content, they bring nutritional and economic challenges. Alternative proteins and fats are being developed but remain limited by cost and availability, whereas aquaculture by-products are abundant, inexpensive, and, through enzymatic hydrolysis, offer promising nutritional and functional benefits.

We undertook a study to assess the feasibility of halving the FM content (low fish meal -LFM- vs high fish meal -HFM-) in the pre-grower diet of red sea bream (*Pagrus major*), with or without supplementing with protein hydrolysates derived from aquaculture by-products (shrimp hydrolysate -SH- or tilapia hydrolysate -TH-). We also included stress situations (daily net chasing for 1 minute) to study the interactions between farming stress and diet. After 15 weeks of rearing and the final weighing, the fish were subjected to physiological sampling and a bacterial challenge to better understand the underlying physiological mechanisms.

Although the diet with low FM content yielded very satisfactory zootechnical performance, close to that of the control diet, the addition of hydrolysates accelerated growth while reducing the feed conversion ratio, and FM use (Fig. 1). Stress similarly impacted all diets, reducing feed intake, growth, and health indicators. All these results are supported by variations in intestinal structure, cortisol levels, and the expression of growth factors in the liver. Hydrolysates also reduced sensitivity to bacterial infection in fish fed the LFM diet. These effects appeared to be linked to improvements in several markers of the non-specific immune system.

Overall, this study highlights the great potential of hydrolysates to further reduce aquaculture's dependency towards wild fish and pave the way for future research on feed optimization.

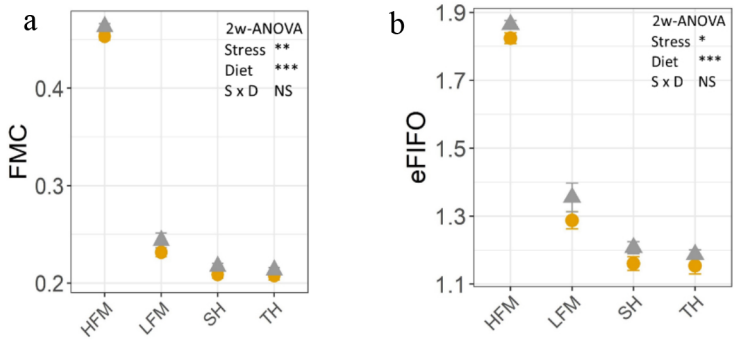


Figure 1. (a) Fish meal consumption (FMC, in kg/kg of produced fish) and (b) economic Fish In Fish Out ratio (eFIFO) of red seabream fed experimental diets for 15 weeks. HFM: High Fish Meal, LFM: Low Fish Meal, SH and TH: LFM supplemented with Shrimp Hydrolysates or Tilapia Hydrolysates respectively.

# ENHANCING AQUACULTURE PRODUCT QUALITY: THE ROLE OF SENSORY EVALUATION IN ASSESSING NUTRITIONAL AND FEEDING STRATEGIES

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Aquaculture plays an important role in global food security and economic development, providing a growing supply of high-quality animal protein. Nowadays, it supplies most of the fish consumed worldwide (FAO, 2024). Among the factors influencing aquaculture production efficiency and product quality, nutrition and feeding strategies stand out for their potential to enhance animal health, to improve sustainability of activity through the reduction of unfavorable environmental impacts and meet consumer expectations. Changes in feed formulation, particularly the incorporation of alternative protein and lipid sources, can significantly alter the sensory quality of aquaculture products, basically with respect to appearance, texture, flavor, taste and odor, which are key drivers of consumer acceptance (Calanche *et al.*, 2019).

Sensometrics found in sensory evaluation and mathematics tools provide effective methods to evaluate the impact of dietary formulations and to guide the development of products that align with market demands. However, many aquaculture nutrition studies are limited by experimental designs that focus on isolated variables, restricting a more comprehensive understanding of how feed formulation influences the overall quality of the final product. The lack of scientific rigor in the application and use of sensory science in the field of aquaculture is a persistent problem today, despite the recognized importance of the sensory quality of fishery and/or aquaculture products in commercial terms. This presentation aims to highlight the role of sensory analysis in nutrition research, emphasizing their value as integrated tools to assess the effects of feed composition and to tailor the sensory quality of aquaculture products.

Sensory evaluation in aquaculture has evolved across four main application areas: nutrition and feeding, production systems, product quality, and marketing. Properly designed sensory studies can be employed to generate objective data or apply hedonic and affective testing to capture consumer perceptions. Sensometrics offers valuable tools to quantify and interpret these responses, allowing researchers to relate to organoleptic properties with nutritional and biochemical parameters. In the agrifood field many researchers often default to using complex statistical methods simply because modern software makes them easily accessible, leading to unnecessarily sophisticated analyses (Qannari, 2017). However, true progress in sensometrics requires grounding research in psychological, behavioral, and motivational concepts that genuinely reflect the phenomena under study. Sensometrics is inherently interdisciplinary, and advancements should be informed by developments in psychology, behavioral and social sciences, neuroscience, and machine learning. With the advent of new data collection techniques, powerful computing, and advanced analytical tools like meta-analysis and interactive databases, sensometrics is well-equipped to address new challenges in understanding perceptions and preferences. Ultimately, there is a strong reason to believe that sensometrics has a bright future, especially in the era of big data.

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# ORAL ADMINISTRATION OF CHITOSAN-ENCAPSULATED BETA-DEFENSIN FAILS TO IMPROVE EUROPEAN SEA BASS IMMUNITY

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**Introduction**

The exponential growth of aquaculture in recent years has led to an increase in diseases caused by opportunistic pathogens (viruses, bacteria, and fungi), resulting in significant economic losses. Among viral pathogens, one of the most relevant in the Mediterranean area is Betanodavirus (NNV), the causative agent of viral encephalopathy and retinopathy (VER) in fish. NNV can cause up to 100% mortality in juvenile stages of susceptible species, including the commercially important European sea bass (*Dicentrarchus labrax*) (Kibenge, 2019). Antimicrobial peptides (AMPs) are key components of the innate immune system and are found across a wide range of organisms. These small molecules regulate inflammation, promote leukocyte recruitment, and bridge the innate and adaptive immune responses. In addition, AMPs exhibit broad-spectrum activity, acting directly against bacteria, viruses, fungi, and parasites by disrupting their membranes. Due to their low likelihood of inducing resistance, AMPs are considered a promising alternative for enhancing disease resistance and improving overall fish health. Among them, beta-defensins (Bdef) have attracted attention for their antibacterial, antiviral, immunomodulatory, and anti-inflammatory properties. The use of AMPs as a preventive measure against NNV is increasingly supported by recent scientific evidence (Valero et al., 2020; Cervera et al., 2024). However, the *in vivo* role of orally administered Bdef in European sea bass has not yet been explored. Therefore, the aim of this study was to evaluate the involvement of European sea bass Bdef1 in NNV infection and its potential as an immunomodulatory agent in functional diets designed for sea bass prior to infection.

**Material and methods**

The synthetic mature peptide Bdef1 derived from European sea bass was encapsulated at 1 mg/mL in alginate-chitosan nanoparticles (NP) using a method that was previously described (Collado-González et al., 2020)we report the synthesis of nanocomposites for the oral delivery of insulin composed of alginate, dextran sulfate, poly-(ethylene glycol. Then, juvenile European sea bass were fed for 5 days with: Control (commercial diet), NP (diet with empty nanoparticles a), and NP+BD (nanoparticles with Bdef1) diets. After sampling for immune analysis, the NP and NP+BD groups were infected with a sublethal dose performed by intramuscular injection of 50 µL of the containing $10^4$ TCID50 of NNV/fish. The control group was injected intramuscularly with 50 µL of PBS alone. At 2 and 24 days post-infection (dpi), samples of brain, head-kidney and hindgut of each fish were collected and processed for immune gene expression analysis by qPCR. Moreover, serum samples were collected to detect NNV specific antibodies using a ELISA assay and as well to evaluate the antibacterial activity agains *Vibrio harveyi* and *Vibrio anguillarum* using a method previously described (Oriol Sunyer & Tort, 1995). Data were expressed as the % of bacterial growth inhibition.

**Results**

Our results showed that sea bass fed with encapsulated Bdef1 during 5 days had not changed their immune parameters. In addition, the antiviral response elicited against NNV was again not altered. In fact, the results obtained from the ELISA assay performed on serum samples have shown that the production of specific antibodies was reduced by the intake of Bdef1.

**Discussion and conclusions**

This study suggests that 5 days dietary administration of encapsulated Bdef1 exerts a limited effect on the immune response of European sea bass as well as little response during NNV infection. Bdef1 appeared to modulate the immune response without inducing excessive activation. Additionally, a significant increase in antibacterial activity was observed against *Vibrio harveyi*,

while no effect was detected for *V. anguillarum*. ELISA results showed lower virus-specific antibody levels in treated fish, indicating a potential modulation of the adaptive immune response. Overall, the promised benefits of Bdef1 as an immune-modulating agent are controversial, supporting further research to optimize its delivery and application in aquaculture.

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# CIRCULAR BIOECONOMY IN AQUACULTURE: INNOVATION FOR SUSTAINABILITY IN ARID ZONES

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The adoption of circular bioeconomy principles in aquaculture represents a key strategy for achieving sustainable development in resource-constrained regions such as La Guajira (Colombia). Based on recent research on intensive tilapia farming (*Oreochromis niloticus*) and low-impact aquaculture production models (Zimmerman et al., 2023; FAO, 2024), this study evaluates the viability of Recirculating Aquaculture Systems (RAS) as an innovative solution to enhance productivity and reduce environmental impacts. Through case studies in Riohacha and Fonseca, RAS proved to reduce water footprints, improve food security, and generate stable income for rural communities. The proposed model aligns with Sustainable Development Goals (SDGs 2, 6, 12, and 14), offering a replicable framework for arid areas in Latin America.

## Introduction

La Guajira faces severe environmental constraints for aquaculture, characterized by annual rainfall below 500 mm, high evaporation rates, and aquifer salinization (IDEAM, 2023). Conventional aquaculture systems generate between 15 and 20 $m^3$ of effluent per ton of fish, negatively affecting freshwater bodies.

Circular bioeconomy seeks to close water and nutrient cycles through sustainable practices such as RAS and multitrophic systems (Chopin, 2022). This approach integrates technology, environmental management, and resilient food production in water-scarce settings. Table 1 summarizes the main circular bioeconomy strategies applied in aquaculture:**Methodology**

Two RAS pilots were evaluated, integrating:

- **Biofloc technology:** Conversion of nitrogenous waste into microbial biomass
- **Aquaponics:** Use of effluents to cultivate *Oreochromis niloticus*
- **IoT sensors:** Real-time monitoring of pH, oxygen, and temperature

**Table 1. Main circular bioeconomy strategies applied in aquaculture**

| Strategy | Description | Reference |
|---|---|---|
| Water use optimization | RAS with ≥90% recirculation, reducing dependence on external sources | Dauda et al. (2020) |
| Functional feeds | Use of diets with probiotics and prebiotics to improve feed conversion | Hai (2020) |
| Waste valorization | Integration of biofloc and bio-RAS to convert sludge into secondary biomass | Emerenciano et al. (2021) |
| Renewable energy | Incorporation of solar energy to reduce carbon footprint | Nhan et al. (2023) |

*(Continued on next page)*

## Results

The case studies in Riohacha and Fonseca showed that implementing RAS under circular bioeconomy principles reduced water consumption by 40% compared to traditional systems, significantly contributing to water sustainability in arid zones. Furthermore, consistent production of 8 tons per hectare per year of tilapia was achieved during drought seasons, enhancing local food security. Economically, rural incomes increased by 35% through direct sales in regional markets.

## Discussion

The integration of RAS and circular bioeconomy technologies reduces pressure on aquifers and surface water bodies—crucial in arid regions. Additionally, the use of functional feeds improves feed conversion, lowering production costs (Hai, 2020). However, the main barrier identified was the high implementation cost (USD 15,000/ha), which can be mitigated through state incentives and public-private partnerships. The use of renewable energy represents a complementary strategy to further reduce operational costs.

## Conclusions

Implementing RAS under a circular bioeconomy approach in La Guajira can:

- Generate 1,200 direct jobs over the next five years.
- Produce more than 1,500 tons per year of sustainable animal and plant protein.
- Reduce pressure on local aquatic ecosystems by 75%.
- Increase the socio-economic resilience of rural communities.

This model has strong replicability potential in other arid regions of Latin America, contributing to SDG achievement and promoting innovation in sustainable aquaculture.

## Acknowledgments

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# SUSTAINABLE BIOCHAR ADDITIVES IN AQUAFEEDS: EFFECTS ON METABOLIC PERFORMANCE IN TURBOT (*Scophthalmus maximus*)

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## Introduction

Biochar is a carbon-rich material produced through the pyrolysis of biomass under low-oxygen conditions. It is a highly product with a wide range of applications, among which its use as a dietary additive in aquaculture has shown promising potential. Its porous structure and high retention capacity have been linked to enhanced nutrient absorption, thereby improving feed efficiency and overall fish health (Amjad et al., 2024). Additionally, these retention characteristics may contribute to trapping nitrogenous compounds, diminishing its presence in water (Khalid et al., 2024). Beyond its functional benefits, the use of biochar derived from wood waste aligns with circular economy principles, supporting sustainable production while potentially reducing the need for other feed ingredients such as fishmeal and fish oil. In this context, the objective of this study was to evaluate the metabolic effects of dietary biochar supplementation in turbot (*Scophthalmus maximus*), with a particular focus on determining an optimal inclusion level without impairing fish growth or health.

## Material and methods

The biochar was produced on an industrial scale through the carbonization of plum (*Prunus domestica*) residues at around 450 °C. A total of 315 turbot juvenile (initial mean body weight of ~34 g) were randomly distributed into 9 tanks (35 fish per tank in triplicate). They were fed manually *ad libitum* twice daily with three different diets: i) control diet (CT), ii) diet supplemented with 0.25 % biochar (B0.25) and iii) diet supplemented with 0.50 % biochar (B0.5). Fish were fed experimental diets for 65 days at the Marine Aquaculture Facility "El Bocal" of the Spanish Institute of Oceanography in Santander. An intermediate biometric sampling was conducted to monitor growth progression. At the end of the feeding trial, final biometric measurements were recorded, and plasma, liver, and muscle samples were obtained. Somatic indexes were calculated, and cortisol along with metabolic parameters were analysed in each tissue.

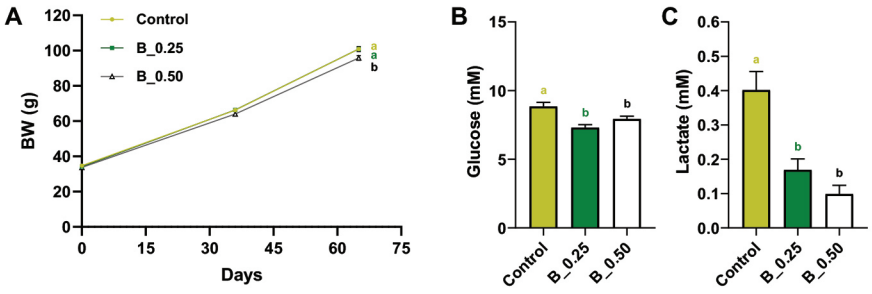


**Fig.1: A)** body weight (BW) evolution during the experiment; plasma glucose **B)** and lactate **C)** levels at the end of the experiment.

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**Results and discussion**

The inclusion of 0.25 % biochar in the diet did not impair growth compared to the control group; however, the 0.50 % inclusion negatively affected performance, resulting in significant lower final body weight and body length. Viscerosomatic index (VSI) was significantly reduced in fish fed the B0.5 diet. Since no differences were found in the hepatosomatic (HSI) or intestinal length (ILI) index, this reduction in VSI may indicate lower visceral fat accumulation, possibly reflecting increased energy expenditure. This energy could be used to compensate some physiological stress generated by an excessive inclusion level of biochar.

Fish fed with biochar reduced plasma energy metabolites (proteins, glucose, TAG, cholesterol and lactate) compared to the control, although all levels were within normal physiological range. In B0.25 fish group, these results together with the maintained growth and non-significant trends found in liver and muscle, suggested a better metabolic efficiency, probably due to more effective nutrient utilization and reduced basal energy mobilization requirements. In contrast, in fish fed B0.5 diet, these reduction of circulating metabolites accompanied by impaired growth and the tendencies followed in the tissues, could be indicative of an energy metabolic resources redirected toward maintenance or detoxification, rather than growth. This suggests a mild adaptive response, perhaps due to an overdose of biochar inclusion in the aquafeed.

The significant decrease in plasma lactate observed in specimens fed with biochar diets reflected a reduced dependence on anaerobic glycolysis or better tolerance to acute stress. The tendency towards an elevation of cortisol with biochar, without exceeding low physiological levels, suggested that the additive may cause mild endocrine adaptations, but without inducing a chronic or harmful state of stress (at least in fish fed with an intermediate level of biochar).

These results indicate that dietary biochar, particularly at 0.25 % inclusion level, may contribute to more efficient metabolism and energy utilization without compromising growth performance or fish health. Combined with its potential for reducing environmental impact, biochar represents a promising and sustainable additive for aquafeeds.

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# IMPACT OF COPPER ON THE INTERACTION BETWEEN THE OYSTER *Magallana gigas* AND THE BACTERIAL PATHOGEN *Vibrio aestuarianus*

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## Introduction

The widespread use of copper as a biocide in French vineyards has led to the accumulation of heavy metals in coastal environments, raising concerns about its ecological impacts. Oysters (*Magallana gigas*), known to be among the strongest marine bioaccumulators of copper, can accumulate concentrations ranging from 719 to 14,000 µg/g of dry weight. While copper is an essential micronutrient required for enzymatic functions, elevated levels can exert strong selective pressure on microbial populations, promoting the evolution of metal-resistance mechanisms.

*Vibrio aestuarianus* subsp. *francensis* is an emergent marine pathogen first described in France in 2001, with a re-emergence observed in 2011–2012. It is now widely distributed along French and some other European coasts[1-2]. This pathogen is responsible for mass mortality events in market-size oysters, causing real and growing economic losses in aquaculture. *V. aestuarianus* subsp. *francensis* strains are clonal and characterized by oyster-specific pathogenicity and a notable copper-resistant phenotype compared to the environmental non-virulent strains of the species[3]. Copper resistance is also observed in other oyster pathogens such as *V. tasmaniensis* and *V. crassostreae*, suggesting a broader evolutionary trend linked to host adaptation[4-5].

In oysters, copper homeostasis is closely tied to immune function. Mechanisms such as the regulation of metallothioneins and metal transporter genes (e.g., Ctr1, ATP7A, ZIP1, Znt2) contribute to resistance against other vibrio species infections[4;6]. These findings underscore the complex interplay between environmental metal exposure, pathogen evolution, and host immune responses in marine ecosystems. However, the immune response of adult oysters during *V. aestuarianus* pathogenesis and the role of environmental factors such as copper levels remains poorly understood. This study aims to investigate whether accumulated copper influences the outcome of the host–pathogen interaction, by deciphering copper-induced immune modulations in oysters and their impact on disease progression.

## Materials and methods

Adult *M. gigas* oysters were exposed to copper ($CuSO_4$ 0, 30, 60 and 120 µg/L; 3 groups of 40 animals per condition) via immersion for 1 month. 15 oyster per copper condition were collected at 0, 14 and 28 days to quantify copper content and determine microbiota composition in order to evaluate correlations with disease progression. Subsequently, the osyters were bath-challenged with *V. aestuarianus* strain 12/016 or in the absence of bacteria (control group) (3 replicates of 10 animals per condition) to assess the impact of copper pre-exposure on infection outcomes. Mortality was monitored daily during 14 d. Hemolymph was collected before and after infection for downstream analyses (hemocytary titre, ROS production, and bacterial multiplication). Finally, gills were collected for transcriptomic analysis by RNAseq. To assess immune modulation and copper-mediated protection to the infection, gills (n=5) from infected and non-infected animals were dissected pre- and post-infection before mortality peak started. Samples were used to extract RNA and sequencing. Differential expression analysis was performed.

*(Continued on next page)*

**Results**

Copper pre-exposure conferred a moderate yet reproducible protective effect against *V. aestuarianus* infection in vivo, with reduced mortality observed across independent exposure and infection protocols. Copper dosage data suggest a potential correlation between individual copper content and mortality timing. Our results also revealed that copper pre-exposure did not impact *V. aestuarianus* growth in plasma either in vitro or in vivo, nor did it affect baseline hemocyte populations. However, transcriptomic (RNA-seq) and targeted qPCR analyses are ongoing to evaluate whether copper modulates the expression of immune-related genes in gill tissues and hemocytes. Notably, preliminary observations suggest that copper pre-exposure may reduce intracellular survival of *V. aestuarianus* in oysters' hemocytes. This potential enhancement of cellular immunity remains to be validated through further experiments. In parallel, to further investigate the physiological consequences of copper exposure, microbiota analyses are currently underway to determine whether copper alters oyster microbial communities.

**Discussion and conclusion**

Our findings suggest that copper accumulation in oysters may contribute to modulating the host–pathogen dynamic during *V. aestuarianus* infection. The observed moderate protection conferred by copper pre-exposure supports the hypothesis that metal-induced immune priming plays a role in disease outcome. While copper did not affect pathogen growth (neither in vitro nor in vivo) or baseline immune cell populations, reduced intracellular bacterial survival in hemocytes of copper-expossed oysters' points toward copper-mediated enhanced cellular immune defenses. These results align with previous reports linking heavy metal homeostasis to antimicrobial activity in oysters. Ongoing microbiota analyses and localization studies will help clarify whether copper's impact is mediated by host immunity alone or also involves shifts in microbial communities. Taken together, this work highlights the complex interplay between environmental metal exposure, immune modulation, and pathogen success—emphasizing the need to consider abiotic stressors in the management of oyster health in aquaculture settings.

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# DOMINANT *Vibrio harveyi* SEROTYPES PATHOGENIC FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*) IN THE IBERIAN PENINSULA

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## Introduction

*Vibrio harveyi* is one of the major agents of vibriosis in marine aquaculture, causing considerable economic losses globally (Austin & Zhang, 2006). Moreover, global warming is causing a shift in its epidemiological role. In the last decade, *V. harveyi* has been increasingly associated with outbreaks in farmed European sea bass (*Dicentrarchus labrax*) along the Mediterranean Sea, posing a serious threat to the industry. The pathogenic strains are not serologically homogeneous and there appear to be three dominant serotypes among the Mediterranean ones (Amaro et al., 2020). However, scarce research comparing their characteristics exists. That is why, despite vaccination efforts targeting *V. harveyi*, outbreaks continue to occur, suggesting that vaccine efficacy may depend on the specific serotype used in its formulation. Thus, *V. harveyi* represents an evolving threat in Mediterranean aquaculture systems and underscores the need for updated surveillance and control strategies. In this study, we aimed to study the serological variability and their potential immunogenicity (based on outer membrane proteins (OMPs) and lipopolysaccharide (LPS) and O-antigen characteristics).

## Materials and methods

Bacterial strains: a collection of strains isolated from diseased seabass in farms located in the Iberian Peninsula was identified using PCR targeting the *V. harveyi toxR* gene (Pang et al., 2006). All strains were routinely grown on Tryticasein Soy Agar or Broth supplemented with 1 % NaCl (TSA-1 or TSB-1) at 28 ºC.

Serology: rabbit polyclonal antisera were raised against formalin-killed whole cells of strains representative of the three putative dominant serotypes (ser): 972 (ser A), 203 (ser B) and C2 (ser C). Then, slide agglutination with whole cell suspensions ($10^8$ colony forming units (CFU)/mL) and O-antigens in PBS was performed (Fouz et al., 1992). Preparation of LPS and OMPs: representative strains of each serotype (see above) were selected for cellular envelope characterization. Crude fractions of LPS were obtained from $10^8$ CFU/mL from overnight TSB-1 cultures and OMPs were extracted as previously described (Fouz et al., 1992).

Electrophoretic analysis and immunoblot: LPS and OMPs were separated by SDS-PAGE with a 10% acrylamide separation gel. In the case of OMPs protein bands were stained with Coomassie brilliant blue. For immunoblotting, fractioned LPS or OMPs were transferred to a PVDF membranes (100V/1 h) that were probed with polyclonal sera anti ser A, B or C diluted 1:750 and, subsequently with anti-rabbit IgG-AP-conjugated (Sigma) diluted at 1:3000. Bands were visualized using NBT and BCIP.

## Results and discussion

Isolates from diseased seabass were confirmed as *V. harveyi* by using specific PCR. Agglutination tests showed that they mostly belonged to ser A and C and to a lesser extent to ser B, previously reported in Croatia (Pavlinec et al., 2022). Moreover, no crossed reactions were observed among serotypes.

LPS patterns differed among serotypes, with ser B and ser C harboring more medium molecular weight O-antigen bands and lipid A (signal below 35 kDa) than ser A (Figure 1). As occurred in agglutination assays, no crossed reactions among serotypes were observed.

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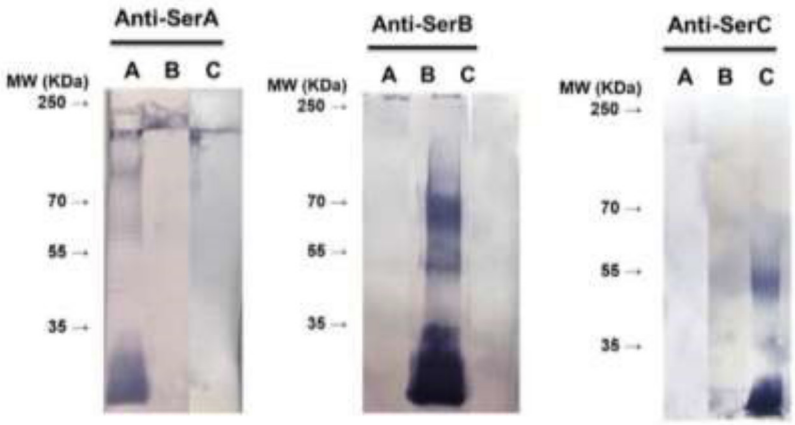


Figure 1. Immunoblot analysis of lipopolysaccharide (LPS) of *Vibrio harveyi* using polyclonal antibodies raised against serotypes (Ser) A, B and C. Lanes: LPS from SerA strain (972) (A), SerB strain 203 (B) and SerC strain C2 (C). Immunostaining was performed with primary antibodies anti-*V. harveyi* SerA, SerB or SerC (1:750), and anti-rabbit IgG-AP-conjugated (1:3000), and visualized with NBT and BCIP.

Regarding the OMPs patterns, all strains exhibited common protein bands, with few serotype-specific ones (data not shown). OMPs immunoblots reinforced these observations, since strains of the three serotypes reacted with antibodies raised against different serological groups. In fact, low molecular weight bands (30-40 kDa) were common in strains of the three serotypes (data not shown). If these common immunogenic OMPs were protective, vaccines developed with strains belonging to any serotype could confer some protection against others.

Our results are especially relevant for the future development of broadly protective and effective vaccines against this new concerning pathogen. Moreover, the emergence of new serotypes highlights the potential lack of cross-protection and immunogenic overlap between circulating and vaccine strains.

Conclusion

This study highlights the serological diversity of *V. harveyi* strains responsible for vibriosis in European sea bass. For this reason, current vaccination strategies may not provide broad protection, reinforcing the importance of continued monitoring and the development of more comprehensive vaccines.

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**Acknowledgements**

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# APPLICATION OF ECOTOXICOLOGICAL AND GEOCHEMICAL TECHNIQUES IN MARINE SEDIMENTS

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**Introduction**
Dredging involves the relocation of marine sediments for different purposes such as bioremediation or maintenance of ports and navigation channels (Beljin et al., 2023; Eke et al., 2023). Although it is currently an essential activity, dredged sediments are classified as hazardous waste due to the amount of contaminants, such as Potentially Toxic Elements (PTEs) including As and Pb (DCMD, 2021; Martínez-López et al., 2021; Beljin et al., 2023; Yi et al., 2023). Consequently, recent research focuses on reducing pollutant concentrations to enable the reuse of dredged sediments in different areas such as construction and agriculture (Rozas et al., 2018; Loudini et al., 2020; Beljin et al., 2023).

The main objective of this work was to characterize dredged sediments from Los Nietos, a highly environmental impacted area in the Mar Menor coastal lagoon. Subsequently, the study analysed the risks associated with As and Pb to public health and ecosystems for the subsequent use of the dredged material in beach regeneration and the creation of construction materials.

**Materials and methods**
Properties and mineralogical composition of 20 marine sediment samples were analyzed according to the current Spanish regulation guideline, "Directrices 2021"(DCDM, 2021). Then, bioaccessibility of PTEs was determined by *in vitro* digestion to determine human health risk associated with As and Pb according to current standardized regulations (USEPA, 1989). Finally, an ecotoxicological study was made to evaluate As and Pb transference from dredged sediments to the aquatic ecosystem.

**Results and discussion**
The results showed that the highest total and bioaccessible concentrations of As and Pb were found in the closest areas to ports and watercourses, which were considered sources of pollution related to anthropogenic activities such as maritime transportation, agriculture and urban development (Ardila et al., 2023). These samples were classified as hazardous sediment according to DCDM (2021) and were associated with unacceptable risk to human health according to USEPA (1989) as well as with evidence of transference of As and Pb to aquatic ecosystem. Therefore, these dredged materials cannot be used in beach regeneration or creating construction materials.

**Conclusions**
This work highlights the importance of marine sediments geochemistry in assessing human and environmental health risks associated with PTEs, particularly when considering new productive uses of marine sediments.

**Acknowledgements**
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# FIRST STEPS FOR THE USE OF SALTPANS IN THE RESTORATIVE AQUACULTURE OF THE NATIVE EUROPEAN OYSTER

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## Introduction

Restorative aquaculture pretends to address the growing demand of food production while delivering net ecological benefit to ecosystems along with social and economic advantages (Alleway et al. 2023). This approach prioritizes the cultivation of low-trophic-level aquatic organisms whose biological functions enhance ecosystem processes such as nutrient cycling, water filtration, and habitat provision. This statement is the cornerstone of the Mar Menor Oyster Initiative (MMOI), an initiative which aims to use bivalves as Nature Base Solutions (NbS) to improve the Mar Menor lagoon (MM) condition. The MM is the largest hyperhaline lagoon in the Mediterranean basin and since the 1970s has been increasingly threatened by a eutrophication process. The lagoon ecosystem collapsed in 2016 as a result of a phytoplankton bloom which caused biodiversity loss, hypoxia and the collapse of the ecological structure (Ruiz et al. 2020). The MMOI has focused on the recovery of the European native oyster (*Ostrea edulis*) to establish a restorative aquaculture program to leverage the ecosystem services provided by the species (Albentosa et al. 2023). This species established a large population in the lagoon of over 100 million individuals in the 1980s and 1990s that has been disappearing, partly due to ecosystem degradation (Rosique 2006). As a first step in the initiative, an oyster culture is taking place in a saltpan located in the north of the MM. In the present work we present two years of growth and survival of the oyster culture in the saltpan.

## Material and methods

*O. edulis* individuals were obtained by breeding lagoon's broodstock at the hatchery (Hernandis et al. 2023). In March 2023, a total of ≈5800 seed were translocated and maintained during 24 months in the entrance canal of the saltpans from San Pedro del Pinatar, located in the north basin of the MM. Initially, oyster height size was 22.24 ± 0.70 mm and live weight was 1.51 ± 0.10 g. The oysters were distributed in circular oyster's baskets stacked in groups of 5. A total of 3 – 6 groups (15 – 30 baskets) were hold, depending on the number and size of the oysters throughout the experiment, in order to maintain a suitable density. Temperature was constantly monitored in the canal throughout experiment.

Once a month approximately, the total live weight of all the oysters was measured and three subsamples of 50 individuals were collected to check for the percentage of mortality and measure size. Besides, a complete biometry with dissections was done in 40 individuals, measuring height, length, width, live weight and dry weight.

## Results and discussion

The maximum temperature recorded in the canal was 34ºC. During the first summer (2023), a high mortality of 38% of the individuals was observed. During the second summer the mortality increased up to 50%. Shell growth followed a seasonal pattern, with higher increases from spring to late summer and lower during the colder months, except for the summer, when shell growth continued while meat weight decreased. Meanwhile, oysters' meat weight followed a similar pattern, but showing a decrease in weight during the warmer months. Condition index followed a similar pattern, probably affected also but the higher temperatures but also the reproductive season, which usually occurs in spring-summer for the MM.

## Acknowledgments

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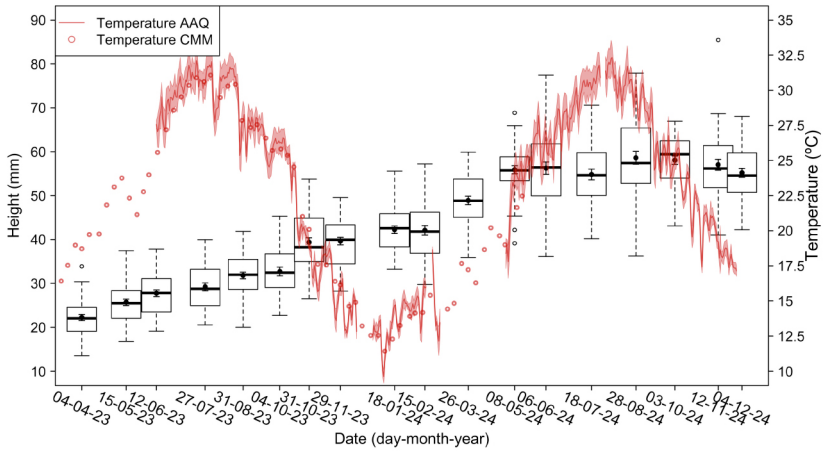


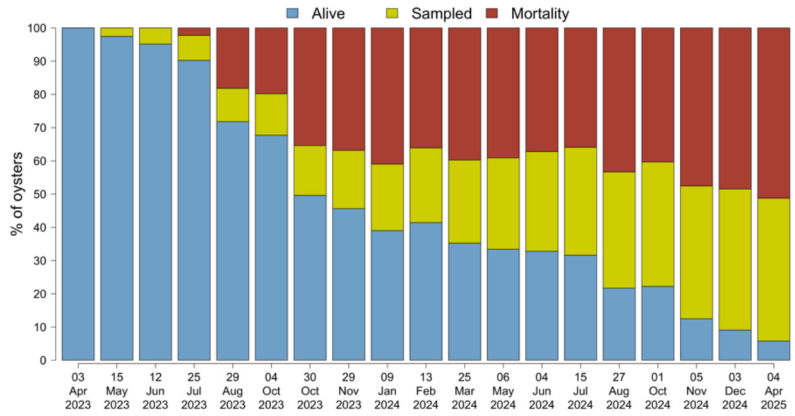


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# IMMUNOMODULATORY POTENTIAL OF A WHOLE PLANT ADDITIVE AT *IN VITRO* AND *IN VIVO* CHALLENGE TRIALS

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In aquaculture, maintaining optimal fish health and welfare is one of the main challenges, primarily due to multiple stressors including high stocking densities, temperature fluctuations, jellyfish blooms, or pathogenic diseases. Among these, infectious diseases notably impact fish survival and overall welfare, leading to significant economic losses. An innovative approach to mitigate these effects and enhance aquaculture sustainability involves incorporating functional feed additives into fish diets.

In recent years, numerous functional feed additives such as probiotics, prebiotics, nucleotides, single-cell proteins, and phytochemicals have been integrated into aquaculture feeds to enhance fish robustness under stressful conditions. Notably, whole plant materials, owing to their phytochemical complexity, have emerged as promising ingredients with potential health benefits.

In this context, we evaluated the immunomodulatory effects of a specific plant material in fish, using both *in vitro* and *in vivo* approaches. Initially, we performed a series of *in vitro* experiments employing rainbow trout cell lines (RTS11 and RT-gut) and primary head kidney leukocyte cultures. Here, we examined the expression of inflammatory biomarkers following stimulation with *Aeromonas salmonicida* in the presence of the plant extract. The results indicated a significant reduction in the expression of pro-inflammatory genes, including *il1□*, *tnf-□*, and *saa5*. Additionally, flow cytometry analyses revealed a decrease in phagocytic capacity of RTS11 cells, and reduced IgM secretion by B cells in primary cultures. These outcomes suggest differential immunomodulatory effects depending on cell type and the concentration of plant material.

Encouraged by these findings, we subsequently formulated an experimental diet incorporating the tested plant material combined with other functional ingredients, aiming to evaluate potential synergistic effects. Two *in vivo* challenge trials were conducted, involving bacterial (*Yersinia ruckeri*) and viral (viral hemorrhagic septicemia virus) pathogens. Remarkably, fish fed with the experimental health-promoting diet showed significantly increased survival rates compared to those fed a control diet or a similar diet without the plant additive.

Collectively, these findings demonstrate that this novel plant additive exhibits promising anti-inflammatory properties and potential synergy with other functional ingredients, making it a valuable addition to functional diets aimed at improving fish health and resilience in aquaculture systems.

# SMALL PARTICLES, BIG IMPACTS: ANOREXIGENIC AND ANXIOGENIC EFFECTS OF MICROPLASTICS IN GOLDFISH

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## Introduction

Microplastics, defined as plastic particles ranging from 1 µm to 5 mm, originate from the fragmentation of larger plastics or from industrial sources such as cosmetics and synthetic textiles. These pollutants pose a serious threat to aquatic organisms, potentially affecting their growth, health, and welfare by causing physiological and behavioural disturbances (Santos *et al*., 2022). This study aims to evaluate the impact of microplastic exposure on energy balance, stress-related behaviours, and overall performance in a freshwater species.

## Materials and methods

Twenty-four goldfish (*Carassius auratus*) were divided into two experimental groups (n=12/group): control group (no exposure to microplastics) and microplastics-exposed group (15 µm polystyrene microspheres, 0.5 mg/L). To maintain microplastic concentration, they were renewed every 24 hours, with a 50% water change over a 14-day period. Food intake, locomotor activity, body weight, and length were daily recorded. After one week of treatment, black-white and open-field preference tests were performed to assess anxiety-like behaviours. At the end of the 14-day exposure, oxygen consumption ($MO_2$) was measured through 24 h. Lastly, fish were euthanized (MS222, 400 mg/L), and liver, intestine, gonads, perivisceral fat, and hypothalamus were collected. The expression of genes related to feeding regulation was analysed in gastrointestinal tissues and hypothalamus.

## Results and discussion

Exposure to microplastics for 14 days significantly reduced both feed intake (Fig. 1a) and food anticipatory activity (FAA), measured during the 2 h preceding feeding time (Fig. 1b). These results suggest a potent anorexigenic effect of microplastics, consistent with previous studies in the same species following 28 days of exposure (Zhang *et al*., 2024). Our results demonstrate that such effects become noticeable after only 14 days of exposure and might be mediated by upregulation of anorexigenic-related genes alongside a downregulation of orexigenic neuropeptides in hypothalamus. Regarding energy expenditure, an increase in metabolic rate was observed (Fig. 1c), aligning with previous findings in black rockfish (*Sebastes schlegelii*; Yin *et al*., 2019).

Together, these findings may explain the observed decrease in both body weight gain and standard growth rate in the microplastics-exposed group (Figs. 2a–b), consistent with previous results in other fish species (Salerno *et al*., 2021). A significant decrease in the hepatosomatic index and a trend towards reduced perivisceral fat index were also observed (Figs. 2c–d); both organs play essential roles in goldfish energy storage.

Moreover, fish exposed to microplastics spent less time and made fewer entries into the aversive zones in the behavioural anxiety tests: i.e. open area in the open field test, (Figs. 3a-b), and the white area in the black-white preference test (Figs. 3c-d). This suggests that microplastics may contribute to heightened anxiety-like behaviour, which may be related to the elevated cortisol levels observed after microplastic exposure (Zhang *et al*., 2024).

In conclusion, short-term exposure to microplastics negatively affected feeding, growth performance, metabolic rate and emotional reactivity in goldfish. These findings highlight the detrimental physiological and behavioural effects of microplastics, compromising energy regulation and overall welfare in this species.

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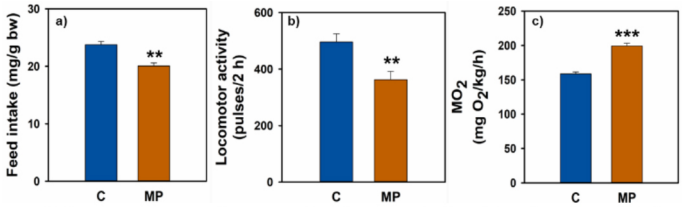


Figure 1. Effect of the exposure of microplastics on a) feed intake (n = 12 days/group), b) food anticipatory activity (n = 12 days/group), and c) oxygen consumption in goldfish (n = 8/group). Mean + standard error is showed. *t-Student* ** $p < 0.01$; *** $p < 0.001$. C: control, MP: microplastics.

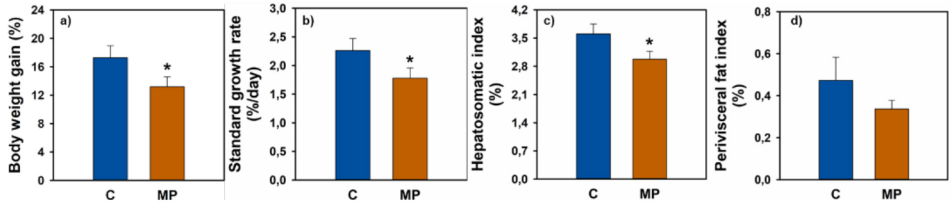


Figure 2. Effect of the exposure of microplastics on a) body weight gain, b) standard growth rate, and c-d) hepatosomatic and perivisceral fat indexes of goldfish. Mean + standard error is showed (n = 12/group). *t-Student* * $p < 0.05$. C: control, MP: microplastics.

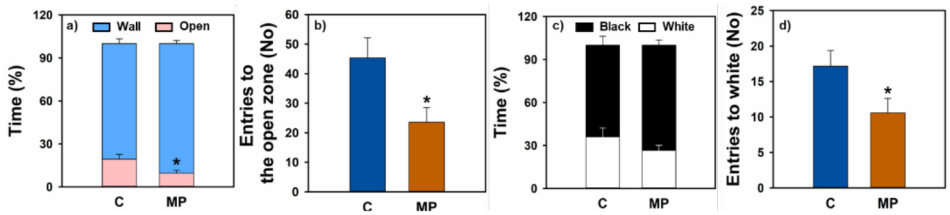


Figure 3. Effect of the exposure of microplastics on a-b) the open field test and c-d) the black-white place preference test in goldfish. Mean + standard error is showed (n = 12/group). *t-Student* * $p < 0.05$. C: control, MP: microplastics.

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# OXYTOCIN SIGNALLING IS REQUIRED FOR SOCIAL REWARD ENCODING IN ZEBRAFISH

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## Introduction

Oxytocin is a key neuropeptide involved in the regulation of social behaviours and the attribution of emotional valence to social stimuli across vertebrates (Dölen *et al*., 2013; Matthews & Tye, 2019). Its signalling is essential not only for social recognition, but also for the formation of social bonds and reinforcement learning based on conspecific interactions (Johnson & Young, 2017). Although the teleost homolog of oxytocin is isotocin, we use the term "oxytocin" here for consistency with the broader vertebrate literature and to emphasize its functional equivalence. Conditioned Place Preference (CPP) assesses whether a stimulus is sufficiently rewarding or aversive to shift preference toward a previously neutral cue (Dölen *et al*., 2013). By comparing pre- and post-conditioning choices, CPP quantifies both the valence and salience of the associated stimulus. This study aimed to evaluate the role of oxytocin in social conditioning and affective processing in zebrafish (*Danio rerio*).

## Materials and methods

We used 36 adult zebrafish (6–8 months) of Tüpfel Long-fin background: 16 oxytocin receptor (*oxtr*) mutants and 16 wildtypes (balanced sexes), plus 4 heterozygotes as social stimuli. Fish were bred and housed under standardized conditions at 27–28 °C with enriched environments and a 14:10 L:D cycle. The *oxtr* line carries a frameshift mutation from a 1-bp TALEN-induced deletion (ZFIN ID: ZDB-ALT-190830–1), confirmed by PCR and sequencing. CPP was conducted in a 4.5 L tank with red and green compartments (visually distinct), and a central grey acclimation zone. Fish were tested individually, with balanced circadian timing and fasted pre-trial. The experiment had three phases: pre-test (5 min acclimation + 10 min preference), conditioning (20 min with a visible, sex-matched, familiar heterozygous stimulus in the non-preferred side), and post-test (same as pre). Time spent in each zone, latency to enter, and number of entries were analysed using BORIS 8.27.10 software All animals were siblings to minimize genetic background effects; tank orientation and water were changed between trials. No significant differences were found between males and females, so data were pooled for analysis. Statistical comparisons between groups were performed using the *Mann–Whitney U test*.

## Results and discussion

Both wild-type zebrafish and oxytocin receptor (*oxtr*) mutants initially showed a strong preference for the green cue, allowing conditioning to be reliably applied in the red compartment. After social conditioning with a visible, familiar conspecific, wild-type individuals exhibited a clear increase in time spent in the previously non-preferred (conditioned) compartment (Figure 1). This shift in preference was absent in *oxtr* mutants, indicating that oxytocin signaling is necessary for associating social stimuli with positive affective value.
Additionally, wild types approached the conditioned zone more quickly after conditioning (Figure 2), reflecting a greater motivational drive towards the socially reinforced cue. In contrast, *oxtr* mutants displayed no consistent directional change in preference, suggesting that while they perceived the social interaction, they failed to assign it a rewarding valence.

These findings support the hypothesis that oxytocin is critical for encoding the rewarding nature of social interactions, through the oxytocin receptor, similar to results from mammals (Dölen *et al*., 2013). Thus, understanding oxytocin's role in social reward can inform strategies to enhance welfare and social cohesion in aquaculture species.

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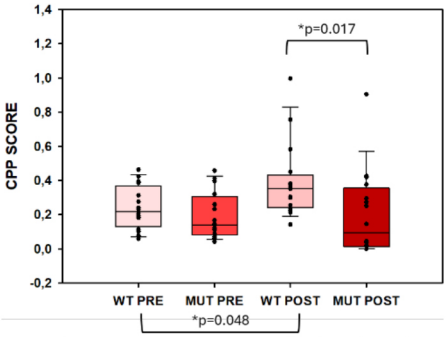


Figure 1. Conditioned Place Preference (CPP) scores calculated as the proportion of time spent in the non-preferred place (NPP) over the total time spent in both compartments: CPP = NPP / (NPP + PP). Boxplots with overlaid bijective dot plots represent individual values per group. *Mann–Whitney U test,* * $p < 0.05$. WT: wildtype, MUT: mutant, PRE: pre-test, POST: post-test.

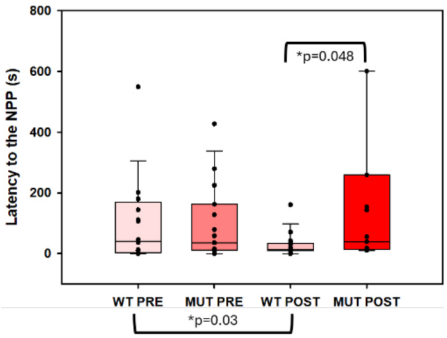


Figure 2. Latency to the non-preferred place (NPP). Boxplots with overlaid bijective dot plots represent individual values per group. *Mann–Whitney U test,* * $p < 0.05$. WT: wildtype, MUT: mutant, PRE: pre-test, POST: post-test.

**Acknowledgements**

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# INNOVATIVE BIOMASS RESOURCES FOR SUSTAINABLE FRESHWATER AQUACULTURE FEEDS: INSIGHTS FROM THE SAFE PROJECT

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## Introduction

The growing demand for sustainable aquaculture feeds calls for novel protein, lipid, and bioactive sources to reduce reliance on fishmeal and fish oil. The Horizon Europe SmartAqua4FuturE (SAFE) project explores diverse biomass resources including insects, microalgae, aquatic plants, and mushrooms produced via circular economic approaches. This work presents the compositional, functional, and safety characterization of these biomasses for integration into freshwater aquaculture diets.

## Material and Methods

Biomasses evaluated included mealworms (*Tenebrio molitor*), redworms (*Eisenia fetida*), microalgae (*Phaeodactylum tricornutum*, *Scenedesmus*), duckweed, watercress, and mushrooms (*Pleurotus ostreatus*). Analytical methods comprised proximate composition (Kjeldahl protein, Soxhlet lipid), β-glucan and chitin quantification, fatty acid profiling (GC-FID), amino acid profiling (UPLC-DAD-MS), elemental and heavy metal analysis (ICP-MS), and bioactive compound quantification (polyphenols, flavonoids, carotenoids via HPLC-UV and colorimetry). Production parameters such as insect diet composition, nitrogen limitation and light exposure for microalgae, and seasonal plant harvesting were adjusted to enhance nutritional traits.

## Results

All biomass types demonstrated favorable nutritional properties and the presence of health-promoting bioactives. Insects provided high-quality proteins and beneficial lipid profiles; microalgae offered valuable long-chain omega-3 fatty acids and carotenoids; aquatic plants contributed minerals, antioxidants, and fiber; and mushrooms grown on alternative substrates retained rich nutrient content. Across all categories, contaminant levels remained within safe regulatory limits, and several cultivation approaches enhanced targeted traits, supporting their suitability for aquafeed inclusion.

## Conclusion

The SAFE project confirms the potential of diverse, sustainably produced biomasses as functional, safe, and nutritionally balanced components of freshwater aquaculture feeds. Their integration could strengthen circular production models, lower environmental impact, and improve the resilience and self-sufficiency of the aquaculture sector.

# GROWTH, SURVIVAL AND HISTOLOGICAL STATUS OF ANTERIOR INTESTINE EURASIAN PERCH *Perca fluviatilis* L. FRY IN DIFFERENT LIGTH CONDITIONS

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**Introduction**

The varying light conditions can significantly affect the efficiency of rearing perch fry in RAS systems by determining fish growth, survival and/or cannibalism. In order to ensure high productivity and reduce production costs, the rearing conditions need to be optimized. Moreover, at an early stage of ontogenetic development, the digestive structures and activities can be affected by the different light conditions (Hamza et al. 2015). In this study, an assessment of the effect of different light regimes on basic growth parameters and selected histological parameters of the middle part of the intestine of Eurasian perch fry was examined.

**Material and methods**

The six-week experiment was conducted with a 42-day-old Eurasian perch (average body weight of 0.055 ± 0.023 g and average body length of 1.78 ± 0.28 cm), where four variants of different photoperiod (L:D – light:dark) i.e. 24L:0D; 20L:4D; 16L:8D and 12L:12D were tested. Throughout the entire rearing period, the intensity of light, measured at the water surface was 800-900 lux, mean temperature of water 21.5±0.5ºC, pH 7.9±0.3, oxygen concentration 8.2±0.6 mg $L^{-1}$. At the end of the experiment 30 perch fry from each group were euthanized, individually weighed, measured and relevant growth parameters were assessed: coefficients of body length variation [CVL(%)]; coefficients of body weight variation [CVM(%)]; Fulton's condition factor [K]. Moreover, cumulative mortality was calculated including the victims of cannibals. For histological examination, the samples of proximal intestine from 30 fish of each experimental group were collected. The tissues were fixed in Bouin's fluid, dehydrated in ethanol, cleared in xylene and embedded in paraffin blocks. Slices 4–5 μm thick were cut using a LEICA RM 2165 rotational microtome (LEICA Microsystems, Wetzlar, Germany) and stained with HE (hematoxylin-eosin) (Zawistowski, 1986). Tthe number of mucous cells within a surface area of 2500 $\mu m^2$ (50 × 50 $\mu$m) and the length of the mucosa folds of the anterior intestine was determined with a LEICA DM3000 transmission light microscope and micro image computer analysis software LEICA QWin Pro (LEICA Microsystems AG, Heerbrugg, Switzerland). The normality of data distribution was tested by the Shapiro- Wilk test and, to confirm for homogeneity of variance, Levene's test was used. The data expressed in percentages were *arcsin* transformed before the statistical analysis. Data were compared using one-way ANOVA. The significance of differences was estimated using a post hoc LSD Fisher test ($p<0.05$). Analyses were performed using Statistica software (TIBCO Software Inc.).

**Results**

At the end of experimental rearing no significant differences ($p<0.05$) between experimental groups for final length and weight of fry as well as for Fulton's condition factor were noted (Table 1). However, significant differences in the values of CVL but not of CVM between perch juveniles reared in different photoperiod regimes were found. Significant differences were also noted in relation to histological parameters, i.e. the number of mucous cells (24L:0D group) and the length of intestinal mucosa folds (24L:0D and 20L:4D groups). The highest survival was observed in the 20L:4D group, while highest intensity of cannibalism was noted in the group reared in 12L:12D (Table 1).

**Discussion**

The lack of significant differences in perch fry growth parameters reared under different photoperiod conditions suggests the limited influence of this factor on rearing efficiency. According to Kestemont et al. (2015) data, at the post-larval stage, the length of the light day did not significantly influence the survival of Eurasian perch. However, the proportion of mortality due to cannibalism was significantly reduced when the day length was increased from LD 8:16 to 24:0, similarly to our results. The higher number of mucous cells and length of intestinal mucosa folds observed in the 24L:0D group may suggest a positive effect of length light day on digestive efficiency in the next stages of perch rearing.

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Table 1. Initial (n=30) and final parameters (n=3 replicates) of Eurasian perch fry according to experimental groups. All data are expressed as mean ± SD. Data in rows marked with different letter subscripts indicate statistically significant differences between groups ($p<0.05$).

| parameter | group / photoperiod | | | |
|---|---|---|---|---|
| | 24L:0D | 20L:4D | 16L:8D | 12L:12D |
| initial body weight (g) | 0.055 ± 0.023 | | | |
| initial total length (cm) | 1.78 ± 0.28 | | | |
| final body weight (g) | 2.90 ± 1.05 | 2.48 ± 1.21 | 2.61 ± 1.22 | 2.60 ± 0.97 |
| final total length (cm) | 5.32 ± 0.66 | 4.99 ± 0.79 | 5.04 ± 0.76 | 4.99 ± 0.60 |
| Fulton's condition factor | 1.46 ± 0.21 | 1.22 ± 0.14 | 1.55 ± 0.21 | 1.56 ± 0.19 |
| CVL (%) | $13.5^{a}$ ± 2.9 | $19.2^{b}$ ± 1.7 | $19.0^{b}$ ± 2.7 | $13.6^{a}$ ± 3.1 |
| CVM (%) | 33.5 ± 8.1 | 32.3 ± 6.9 | 29.4 ± 4.8 | 35.0 ± 3.8 |
| survival (%) | $64.0^{a}$ ± 3.6 | $73.3^{b}$ ± 4.2 | $65.3^{a}$ ± 2.7 | $63.3^{a}$ ± 4.9 |
| cannibalism (%) | $3.3^{a}$ ± 0.6 | $4.0^{a}$ ± 2.8 | $5.5^{a}$ ± 3.0 | $10.7^{b}$ ± 4.6 |
| number of mucous cells | $16^{a}$ ± 6 | $14^{a}$ ± 4 | $11^{b}$ ± 5 | $10^{b}$ ± 6 |
| length of the mucosa folds (µm) | $327^{a}$ ± 123 | $273^{ab}$ ± 156 | $241^{b}$ ± 73 | $234^{b}$ ± 83 |

**Acknowledgment**

This work was performed in the project " Diversification of pond-based production through semi-intensive aquaculture of Eurasian perch, *Perca fluviatilis* " (acronym: PRO-PERCH; grant agreement no 00002-6521.1-OR1400004/17/20), financially supported by Polish Operational Programme "PO RYBY 2014–2020" within European Maritime and Fisheries Fund and Funded by the Minister of Science under „the Regional Initiative of Excellence Program".

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# REDUCING RISK FOR PERSONELL IN NORWEGIAN SALMON FARMING – INTERORGANISATIONAL WORK

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Introduction

Norwegian salmon fish farming companies rely on contractors and suppliers in their production, including crew on specialized vessels such as well boats, slaughter vessels and service vessels. Research indicates that conditions related to interorganizational interaction, such as work pressure and efficiency demands related to economic considerations, can impact personnel safety negatively (Kongsvik et al. 2025), but more knowledge is needed. The interview study presented therefore explores the topic of cooperation and personnel safety further.

The study is part of the ongoing project "Knowledge and measures to reduce risk for personnel in the fish farming industry", financed by the Norwegian Seafood Research Fund (project 902016).

Material and methods

An interview study is being conducted in the period February – august 2025. It includes 15 employees (OHS-personnel at salmon farming companies and contractors, fish farm workers and vessel crew members) who work in companies of different sizes and geographical locations along the Norwegian coast.

An interview guide, which targets risk factors, challenges related to cooperation and collaboration among different actors and possible improvements that can reduce risk for personnel is used.

Results

Results from the interview study will be presented. Key topics include the different types of collaboration agreements between contractors and fish farming companies and how they may impact safety (long term agreements, spot market etc.), prominent risk factors for personnel in operations, how procedures are designed and harmonized between companies, how new types of technologies influence work task and operations (e.g. submerged fish farms), how cooperation operations are planned, performed and evaluated. Furthermore, regulations that influence safety as well as training employees and crew members will be discussed. Based on these results, some suggestions for measures and improvements are given.

**Conclusion**

The study provides knowledge about challenges related to interorganizational interaction in salmon farming and suggestions that can improve risk management and cooperation between organizations moving forward. Results are discussed and communicated to the industry as part of the project.

Literature

Kongsvik, T, Størkersen, K, Thorvaldsen, T. Holen, S. (2025): Between the Devil and the Deep Blue Sea: Work Safety for Contractors in Norwegian Fish Farming, Journal of Agromedicine.

# A STUDY OF TWO ADIPOSE TISSUE DEPOTS IN ATLANTIC SALMON (*Salmo salar)*

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**Introduction**
A growing prevalence of obesity in humans has led to an increased global interest in white adipose tissue biology over recent years. In the aquaculture industry, commercial diets have shifted towards higher lipid content; modern pelleted diets for Atlantic salmon now commonly contain 35 – 38% lipids, compared to early formulations that had lower lipid levels (8 – 22%). The development of these high-energy diets has allowed the aquaculture industry to reduce production time by increasing growth rates and feed efficiency. This change has resulted in higher lipid levels in farmed fish, including larger visceral and myoseptal fat depots, which can cause lower quality filets and physiological changes such as oxidative stress. New insights into the development and function of these adipose tissue depots could aid in preventing excessive fat accumulation in farmed fish.

**Materials and Methods**
This study aimed to compare the morphological and functional differences between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) in farmed Atlantic salmon using both in vivo and in vitro approaches. A combination of cellular characterization techniques was performed, including histology, microarray analyses, and total RNA sequencing, to gain a comprehensive understanding of the unique properties of each adipose depot.

**Results**
The findings revealed significant differences between VAT and SAT. The histological examination showed that VAT was characterized by larger adipocytes, while SAT contained a higher number of smaller adipocytes per area studied. This difference indicates a hypertrophic versus hyperplastic trait in these tissues. VAT adipocytes displayed an enhanced capacity for lipid droplet accumulation and mobilization, highlighting their substantial role in lipid storage.

Gene expression analyses demonstrated distinct profiles between VAT and SAT, affecting insulin resistance, immune activity, energy provision, and lipid metabolism. Notably, VAT had a higher expression of immune-related genes, suggesting increased immune activity and inflammation. Furthermore, SAT predominantly functioned as a direct energy provider, while VAT exhibited greater lipolytic activity, underscoring its dual role as both a lipid storage site and a potential facilitator of free fatty acid mobilization for oxidation in other organs.

In vitro studies reinforced these observations. Primary adipocytes were isolated from VAT and SAT and cultured. Differences in cell morphology were observed, with VAT cells exhibiting a round shape from day 1 of culture, while SAT cells displayed a characteristic fibroblastic appearance and grew in clusters. After inducing differentiation with a hormone cocktail, both cell types changed their morphology and began accumulating lipids. At day 15, the lipid storage capacity of both cell types was evaluated using oil red O and LipidTox staining techniques, demonstrating that adipocytes isolated from VAT had a higher capacity to accumulate lipids than those isolated from SAT.

**Conclusions**
This study provides insights into the distinct morphological and functional characteristics of VAT and SAT in farmed Atlantic salmon. These findings could be crucial in future strategies to reduce excessive fat accumulation and improve fish health and quality in aquaculture.

# 1125
# FEEDING THE FUTURE: SUSTAINABLE LIVE FEEDS FOR AQUACULTURE

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**Introduction**
Modern aquaculture is the fastest-growing animal food sector, yet rearing juvenile fish remains constrained by the lack of suitable live feed. Current feeds such as *Artemia* and rotifers are nutritionally suboptimal, expensive to enrich, and poorly digested by young fish, leading to high mortality, poor growth, deformities, and premature transition to dry feed. There is an urgent need for sustainable, high-quality, immune-boosting live feeds that support antibiotic-free and environmentally friendly aquaculture.

**Methods**
Through the DELIFEED and LiveFishHealth projects, we are developing next-generation live feeds based on Enchytraeids and nematodes. Research activities include:

1. Enhancing essential fatty acid content in novel live feed organisms.
2. Developing industrial-scale cryopreservation methods to ensure long-term storage and global distribution.
3. Evaluating performance in juvenile Atlantic salmon, Atlantic halibut, ballan wrasse, pike perch, turbot, European flounder, and rainbow trout.
4. Investigating live feed potential as vectors for probiotics and oral vaccines, including early vaccination against Rainbow Trout Fry Syndrome (RTFS).

**Results and Discussion**
Preliminary trials show that Enchytraeids and nematodes provide high nutritional value without enrichment, enhance juvenile fish growth by up to 200%, and reduce mortality. They can be mass-produced on organic waste, aligning with circular bioeconomy principles. Potential cryopreservation and anhydrobiosis of live feeds may enable stable supply chains. Early results also indicate promise as oral vaccine carriers, offering a route to reduce or eliminate antibiotics in aquaculture.

**Conclusion**
The integration of novel live feeds into aquaculture has the potential to improve fish survival, growth, and welfare, support sustainable and organic production, and expand the range of species that can be farmed. The DELIFEED and LiveFishHealth projects demonstrate a pathway from innovation to industry adoption, addressing critical bottlenecks in fish larval rearing.

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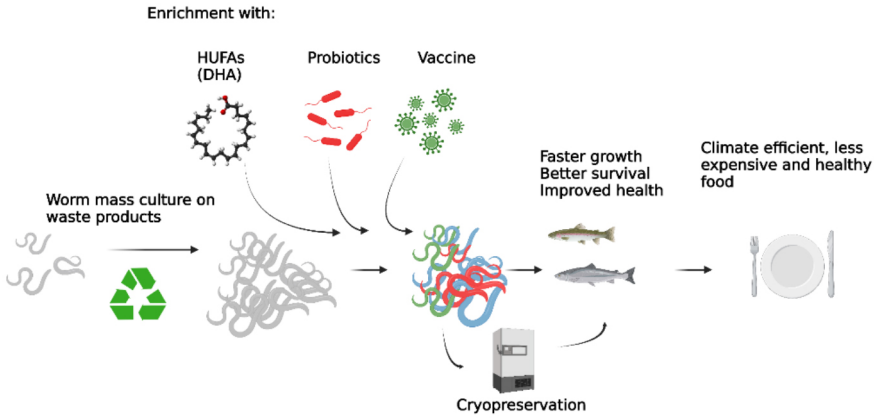


*Figure 1. Conceptual workflow for developing sustainable live feed for aquaculture. Enchytraeids and nematodes are mass-cultured on organic waste products and can be enriched with health-promoting additives, including essential highly unsaturated fatty acids (HUFAs, e.g., DHA), probiotics, and vaccines. These enriched worms are fed to juvenile fish to promote faster growth, improved survival, and better health. Cryopreservation enables long-term storage and stable year-round supply. The approach contributes to climate-efficient, cost-effective, and nutritious seafood production.*

# 1227
# TINY WORMS, BIG POTENTIAL *Enchytraeus albidus* AS STARTER FEED FOR RAINBOW TROUT

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The aquaculture industry is increasingly seeking sustainable feed alternatives to address ecological concerns associated with traditional feed ingredients and enhance the health and growth of farmed fish during their early life stages. Live feed, such as the annelid worm *Enchytraeus albidus,* has been shown to increase growth in marine fish species. In this study, we evaluate *E. albidus* as a potential starter live feed for rainbow trout fry (*Oncorhynchus mykiss*), focusing on growth, health, and fatty acid composition. Fry were fed one of three diets: standard dry feed (DF), live *E. albidus* (EF), or a combination of both (DF/EF) for 21 days, followed by a transition to DF for the following 22 days. Fry initially fed with EF exhibited significantly higher growth rates compared to those on DF, and they maintained superior size even three weeks after being transitioned to dry feed. At the end of the experiment the fish fed EF had a 10% higher mass than fish fed DF. No significant differences were found in health or welfare indicators across the treatment groups, which all demonstrated good health and low mortality. The fatty acid profiles differed between treatments, with lower essential fatty acid DHA (docosahexaenoic acid) levels in fish fed EF. However, despite the lower body levels, critical DHA levels seemed to be preserved in the neural tissues of the fry. These findings highlight the potential of *E. albidus* as a sustainable and effective starter feed for rainbow trout fry. Furthermore, they provide a compelling basis for further investigating the feasibility of *E. albidus* as a bio-carrier for oral vaccines.

# FIT-FOR-PURPOSE ENVIRONMENTAL AND ECONOMIC SUSTAINABILITY ASSESSMENT OF INTEGRATED MULTITROPHIC AQUACULTURE

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## Introduction

Integrated multitrophic aquaculture (IMTA), i.e. co-farming of species from different trophic levels, combines aquatic species into one farming system to provide benefits for: *i)* one or several of the farmed species, e.g. seaweed can grow faster in the nutrient rich waters from fish farming, and/or *ii)* ecosystems, i.e. nutrient uptake from fish farming, and *iii*) aquaculture producers, e.g. better productivity and new revenue streams – combined allowing for production efficiencies as more biomass is produced from the system. This is aligned with societal ambitions on resource-efficiency, circularity and mitigation of drivers for biodiversity loss. Despite the potential benefits, IMTA remains uncommon in Europe, where its application has been mainly limited to pilot-scale trials within the framework of research projects.

In the BLUEBOOST project (https://www.blueboost.eu/), suitable design of IMTA systems will be investigated. Case study experiments are undertaken in Italy, Spain, Portugal, Poland and Brazil and evaluated as to their environmental and economic sustainability. One area of focus is the specific methodological choices needed to assess environmental impacts and benefits of the multi- and circular output nature of IMTA systems. Although some insights have been gained using Life Cycle Assessment (LCA) – feed, effluents and energy use are impact hotspots – a standardized LCA methodology of IMTAs is called for (Hala et al 2024). Further, reduced externalities have so far been underexplored in cost-benefit analysis (CBA) of aquaculture (Knowler et al. 2020).

## Method

An initial LCA literature screening in March 2024, resulted in four studies applying LCA on IMTA (Chary et al. 2020; Mendoza Beltran et al. 2018; Prescott 2017; Wilfart et al. 2020), from which key methodological decisions were extracted, compared and discussed to derive a suitable approach for harmonized evaluation of IMTA systems for BLUEBOOST (Ziegler et al. 2024). Further, we explored the general CBA literature, economic assessments of IMTA and literature on the valuation of environmental externalities relevant in the aquaculture context. Insights gained are important to share and discuss with IMTA experts and LCA practitioners beyond the project for good practice sustainability assessments of IMTAs beyond BLUEBOOST. We have therefore undertaken a more structured systematic literature review in Scopus, identifying additional documents of interest. These are currently in the process of being analyzed to see if and how they add to the findings from the screening and inform on a more comprehensive overview to provide overall good practices when applying LCA to IMTA.

**Table 1** Key LCA decisions and the suggested BLUEBOOST approach.

| Methodological aspect | BLUEBOOST approach |
|---|---|
| **Modelling approach** | Attributional |
| **Functional unit** | 1 kg biomass of economic value |
| **Allocation** | Mass-balanced |
| **System boundary** | Hatchery to farmgate |
| **Impact Assessment** | ReCiPe H + Cumulative Energy Demand |
| **Background data** | Ecoinvent 3.9.1 + Agrifootprint 6.0 |
| **Circularity assessment** | According to ISO 59020 |
| **Nutrient retention** | Bioremediation (N/P/C retained) |

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Here we present the results from the initial screening and the methodology suggested for sustainability assessment in BlueBoost, which is work in progress as additional publications will be analyzed and discussed during spring 2025 for a more generic decision-support framework.

**Results**

The four IMTA LCA studies all focus on different systems and, most importantly, use different LCA method decisions. Functional units varied considerably, i.e. the output of the system which environmental pressures are related to – and which has a strong effect on results. Further, how to allocate environmental burdens between main product and co-products and choice of impact assessment method (and environmental impact categories included) also matter for results on IMTA system performance. Comparability of results between studies is therefore limited without harmonization. The key method choices suggested for BLUEBOOST are found in Table 1.

Drawing on general CBA practices and extant economic assessments of IMTA systems (e.g. da Silva et al. 2022), an applied cash flow calculator is being formulated. In case studies with important environmental externalities, we attempt to identify feasible monetary valuations to be used in the social CBA.

**Discussion**

Robust and comparative LCAs are vital to understand differences between aquaculture production systems and how they can be optimized. A decision-support tool will assist LCA practitioners in future assessments. Alongside, a suitable economic assessment is required to ensure that the proposed solutions are economically viable.

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# MANUAL VS. MACHINE LOADING OF STRAWS FOR THE CRYOPRESERVATION OF COMMON CARP (*Cyprinus carpio*) SPERM

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**Introduction**

Cryopreservation is a useful assisted reproductive technology for the conservation of genetic resources as frozen live cells and tissues can be stored for an infinite period of time. In case of common carp (*Cyprinus carpio*) several frozen gene banks have been created in order to maintain the genetic resources of valuable or disease resistant strains. Commercialization of cryopreservation requires standardization and upscaling of existing techniques. These can include standardization of the sperm-egg ratio used for fertilization of eggs (Nynca et al., 2017; Pataki et al., 2022) and advanced sperm quality assessment methods. In this study we have tested if the use of a commercially available semi-automatic straw loader results in higher post-thaw sperm quality than traditional manual loading of straws. Additionally, we have also investigated if the position of straws during storage (top or bottom goblet on the cane) has an effect on post-thaw quality.

**Materials and methods**

Sperm from 3 common carp males was used in the experiments. Sperm was diluted at a 1:9 ratio in an extender composed of 200 mM glucose, 40 mM KCl, 30 mM Tris (pH 8.0) to which 10% methanol (v/v final concentration) was added as a cryoprotectant. Diluted sperm was loaded into 0.5-ml straws either manually or with an semi-automatic straw loader (SFS, Minitube, Germany). Loaded straws were placed onto dedicated metal frames with 36 positions and frozen in the vapor of liquid nitrogen in a polystyrene box at the height of 3 cm above the level of liquid nitrogen for 3 minutes. Straws were stored in 13-mm goblets attached to aluminum canes immersed in liquid nitrogen. Following thawing in a 40 °C water bath for 13 seconds, sperm quality was determined using a computer-assisted sperm analysis (CASA) system (AndroVision, Minitube, Germany). Altogether, 281 straws were filled with the machine (120 in top and161 in bottom position) and 290 manually (160 in top and 130 in bottom position). Two-way ANOVA was used to investigate the main effects of straw loading methods as well as goblet position on post-thaw motility parameters.

**Results**

Neither the straw loading method ($P = 0.215$), nor the goblet position ($P = 0.116$) had a significant effect on the post-thaw motility of carp sperm (Fig. 1). On the other hand, the loading method had a significant effect on all post-thaw sperm velocity parameters (VCL: $P = 0.006$, VSL: $P = 0.004$, VAP: $P = 0.005$) with machine loading producing higher values than manual filling. Goblet position did not affect post-thaw sperm velocity with the exception of VSL ($P = 0.038$).

**Discussion**

In the current study, we have demonstrated that machine loading of straws for the cryopreservation of common carp sperm results in higher post-thaw sperm velocity values than manual filling. The use of a straw filling machine represents an improvement over manual loading due to increased consistency and faster processing of sperm prior to freezing, leading thus to standardization of existing methodologies.

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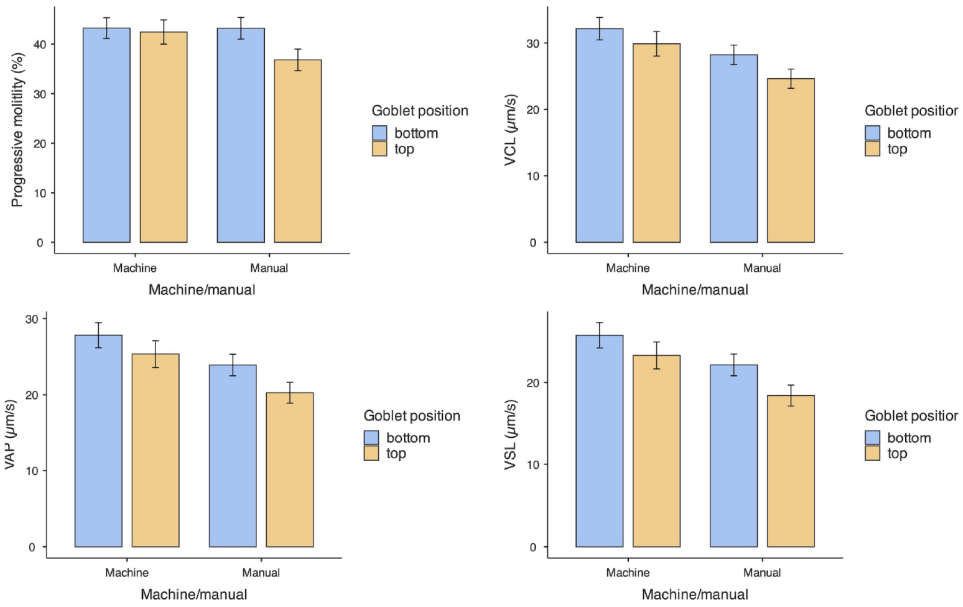


Figure 1. Progressive motility, curvilinear velocity (VCL), average-path velocity (VAP) as well as straight-line velocity (VSL) of cryopreserved common carp sperm loaded into straws either manually or by a machine and stored in bottom or top goblets.

**Acknowledgements**

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# ENHANCED RESILIENCE TO MUCOSAL DISEASES IN ATLANTIC SALMON (*Salmo salar*) SUPPLEMENTED WITH A NOVEL YEAST-BASED INGREDIENT

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## Introduction

Farmed Atlantic salmon frequently encounter harmful infectious and non-infectious factors as well as necessary management practices causing serious mucosal health issues across the industry. Functional feeds are commonly used as preventive measures to enhance fish health and well-fare among which yeast cell wall (YCW) products are well-established. Two independent trials were conducted to evaluate the potential benefits of in-feed supplementation with specific YCW product on two mucosal-associated diseases; Tenacibaculosis (*T. maritimum* challenge) and Amoebic Gill Disease (AGD; *N. perurans* challenge).

## Materials and Methods

Both trials used a baseline recipe reflecting current commercial practices in the European Atlantic salmon industry. Each trial tested 3 diets in quadruplicate as follow. A non-supplemented diet (Control); and two diet supplemented with two novel YCW products (Proto and Y+ respectively; Lallemand, France). Feed were fed to apparent satiation over the trial's duration.

The first trial lasted 15 weeks with fish exposed to *T. maritimum* by immersion at week 5. The second trial lasted 10 weeks with fish exposed to a first AGD challenge at week 5 using upstream shedders; followed by freshwater (FW) treatment and a second AGD challenge. In both trial, growth, feed performance and survival were monitored; visual scoring of the gross pathology (skin lesions and gill score respectively) were performed at interval and mucosal tissue were collected for analysis.

## Results

In the first trial (*T. maritimum* challenge) and compared to the Control; supplementation with Y+ resulted in a significant improvement in survival (+22%; Fig 1A) which was associated with a significant decrease in the proportion of moderate and severe skin lesions (-30% at the end of the trial; Fig 1B). In the second trial (AGD-challenge); diet Y+ was associated with improve specific growth rate and feed conversion ratio (+ 20% and -32% compared to the Control, respectively; Fig 2A) over the trial's duration. Gill scoring over time documented the propagation of the pathology and the positive impact of the intermediary FW-treatment. At the end of the trial, gill-scoring was significantly improved in dietary group Y+ with a marked decrease in the proportion of heavy and advanced scores and, inversely, a higher proportion of fish showing a light AGD-score.

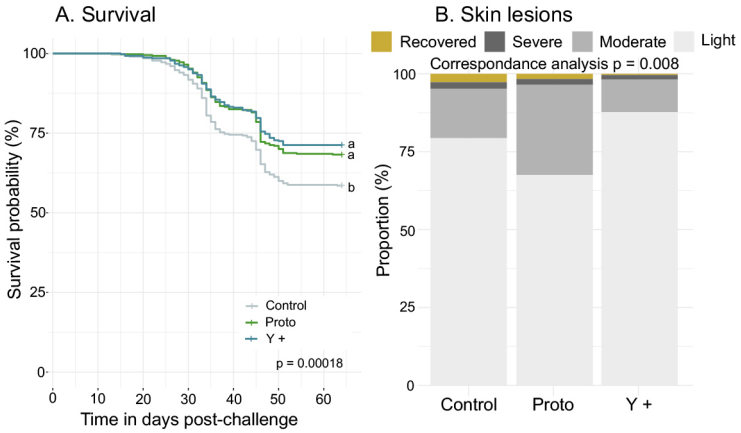


**Figure 1:** A) Survival probability and B) skin score lesions at the end of the first trial (*T. maritimum* challenge). Different letters refers to significant differences.

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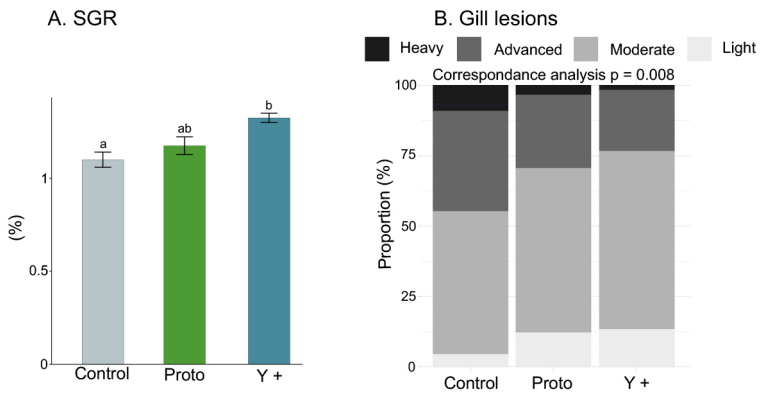


**Figure 2:** A) Specific growth rate (SGR) and B) proportion of gill score lesion's category at the end of the trial (AGD-challenge). Different letters refers to significant differences.

Analyses of histology, gene expression and mucus qualities will be presented to decipher the beneficial effects of the YCW product on mucosal robustness and disease resilience.

**Conclusion**

This novel-YCW product as the potential to contribute to the preventive management of gill and skin health in seawater Atlantic salmon. Further trial's under commercial-like conditions should be performed.

# BEHAVIOURAL AND CARDIAC RESPONSES OF EUROPEAN SEABASS REARED IN SEA-CAGES: INSIGHTS FOR WELFARE STATES ASSESSMENT

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**Introduction**

Aquaculture has grown significantly in recent years, necessitating the development of advanced monitoring techniques to ensure fish welfare and optimize management practices. Understanding how fish respond to environmental and anthropogenic factors is key to improving welfare standards. Surgically implanted biologgers, capable of measuring heart rate (HR) and external acceleration (ACC), are increasingly used to monitor fish behaviour and physiological states, providing valuable insights into activity patterns.

As the aquaculture industry shifts toward more sustainable and ethical practices, welfare monitoring is becoming increasingly important. Within the context of Precision Fish Farming, these technologies facilitate informed decision-making and enhance farm management (Morgenroth et al., 2024; Brijs et al., 2021). Among them, biologgers stand out for their ability to measure HR and ACC, offering insights into fish physiology and behaviour under farming conditions. However, despite extensive research on northern species such as Atlantic salmon (e.g. Brijs et al., 2018), few studies have focused on Mediterranean species such as the European seabass (*Dicentrarchus labrax*).

This study assesses the behavioural and physiological responses of seabass reared in open-sea cages using data from implanted biologgers, applying machine learning techniques to classify welfare-related behavioural states and highlighting the potential of integrating sensor technology and data analysis to support welfare monitoring in aquaculture.

**Materials and Methods**

The study was conducted at the LIMIA-IRFAP aquaculture facilities (Balearic Islands, Spain), using 1000 adult seabass reared in four open-sea cages. Sixteen fish were implanted with biologgers (DST milli HRT-ACT, Star-Oddi®) to monitor HR and ACC over two 15-day periods in March and July. Surgical implantation followed the protocols described by Cabrera-Álvarez et al. (2024), ensuring minimal impact on fish recovery and behaviour. Data were recorded every five minutes and retrieved post-mortem. To classify behavioural states, a Random Forest (RF) model was developed with data obtained from swimming and crowding stress challenges, enabling the definition of four key states: Resting, Regular Activity, Reactive Stress Response, and Proactive Stress Response. The RF model was trained using 540 labelled observations, with independent testing to ensure its robustness. During the swim challenge, fish were exposed to incremental swimming speeds (0.2 to 1.0 m/s), while the stress challenge included progressive stressors such as reduced water levels and net chasing. Behavioural states were labelled as follows: nighttime observations were categorized as Resting, swimming speeds between 0.2-0.6 m/s as Regular Activity, stress-induced peaks as Proactive Stress Response, and post-stress periods as Reactive Stress Response. Following validation, the RF model was used to classify the data in the present study.

All procedures involving fish were approved by the Ethical Committee of Animal Experimentation (CEEA-UIB, Spain; Ref. 206/12/22), the Dutch Central Committee for Animal Experiments (CCD; project AVD40100202115078, 15 November 2021), and the Animal Experiments Committee (DEC) and Authority for Animal Welfare (IvD) of Wageningen University and Research (experiment 2021.D-0005.004). They were performed by trained personnel in accordance with the European Directive 2010/63/UE and Spanish Royal Decree RD53/2013, ensuring optimal animal care, health, and welfare.

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**Results and Discussion**

The biologger data revealed distinct behavioural patterns in seabass under farm conditions. The RF model successfully classified behavioural states with an overall accuracy of 67.38% on the test dataset and over 73% during cross-validation. The model performed particularly well in detecting stress-related behaviours, correctly identifying Proactive and Reactive Stress Responses in over 80% of cases. These stress states were characterized primarily by elevated ACC values, with HR also contributing in reactive contexts.

When applying the RF model to the biologgers data obtained from the seabass reared in open-sea cages, circadian behavioural rhythms that aligned with operational routines were detected. Resting states predominated at night and on weekends when human activity around the cages was minimal. Conversely, stress-related behaviours were more frequent during daylight hours, particularly in July, when water temperatures and maritime traffic were higher. These findings indicate that both environmental and anthropogenic factors significantly influence seabass behaviour and welfare.

Overall, by combining sensor-based monitoring with behavioural classification, this study presents a practical approach to real-time welfare assessment in open-sea aquaculture. The method not only helps identifying stressing events but also provides insights into baseline activity and seasonal activity patterns. These findings underscore the value of integrating behavioural data into farm management strategies and support the development of PFF technologies tailored to open-sea aquaculture systems.

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**Acknowledgements**

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# CARDIAC RESPONSES, ACTIVITY, AND OXYGEN CONSUMPTION OF EUROPEAN SEABASS SUBJECTED TO STRESS CHALLENGES

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**Introduction**

The use of biologgers as tools to monitor behaviour and welfare in cultivated fish is becoming increasingly widespread. These biosensors enable the measurement of physiological parameters such as heart rate and external acceleration, which are related to oxygen consumption during stressful events. In such situations, increased oxygen demand triggers cardiovascular adjustments to maintain metabolic function (Morgenroth et al., 2024).

Assessing swimming ability and both aerobic and anaerobic metabolism is essential for evaluating the physiological state of fish and their ability to cope with stress. Swimming tests and exposure to standardized stressors (e.g., confinement, water level reduction, or net chasing) allow for the determination of the aerobic and anaerobic components of metabolism, which can be quantified through oxygen consumption, heart rate, and external acceleration of the fish. Several studies have linked physiological parameters such as heart rate or acceleration to oxygen consumption during stressful events in aquaculture species of interest, such as amberjack and sea bream (Arechavala-Lopez et al., 2021; Palstra et al., 2024). However, no studies have addressed the relationship between metabolic and cardiac responses to stressful events in European seabass (*Dicentrarchus labrax*), one of the most important species in Mediterranean aquaculture.

The main objective of this study is to characterize the metabolic and physiological activity of sea bass by analysing the relationship between cardiac response, activity, and oxygen consumption in individuals subjected to swimming tests and stress events.

**Materials and Methods**

For this study, 18 adult European bass (WW = 421.1 ± 51.46 g) were housed in a 600L circular tank at a controlled temperature of 22.5 ± 0.45 ºC and fed daily with commercial feed at Wageningen University (WUR). Of these, 12 individuals were implanted with heart rate and acceleration biologgers (DST milli-HRT ACT; Star-Oddi, Garðabær, Iceland). Implantation was carried out under anaesthesia with an aqueous solution of 2-phenoxyethanol (0.3 ml/L), followed by a 10-day recovery period in a quarantine tank.

Swimming tests were conducted in three 127L tunnels, with the water flow speed gradually increasing from 0 to 1 m/s in 0.2 m/s intervals per hour. Heart rate and external acceleration were measured at 10-minute intervals.

For the stress test, the fish were transferred to a 600L tank and subjected to four stressful events, which consisted of lowering the water level in the tank until the dorsal fin emerged, followed by refilling the tank. The events varied in the duration of the time with a low water level and included a net chasing procedure in the fourth event. Recovery periods of 40 minutes were included between events, and heart rate and acceleration were monitored for up to 3 hours after the test.

All experimental procedures were approved by the Dutch Central Committee for Animal Experimentation (CCD), project number AVD40100202115078, as well as by the Animal Experimentation Committee (DEC) and the Animal Welfare Authority (IvD) of Wageningen University.

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**Results and Discussion**

During the swimming test, no significant differences were found in the optimal swimming speed ($U_{opt}$) between the control group and the tagged individuals ($U_{opt}$ Control = 0.737 m/s, $U_{op}$t Logged = 0.740 m/s; p-value > 0.05). Oxygen consumption (MO2) increased exponentially with varying swimming speeds ($R^2$ = 0.461). Additionally, a positive linear correlation was found between oxygen consumption and heart rate, as well as between oxygen consumption and external acceleration as swimming speed increased ($R^2$ = 0.45 and $R^2$ = 0.48, respectively).

In the stress test, an increase in heart rate and external acceleration was detected during the application of the stressors, followed by a decrease in these parameters during the recovery period between events. Heart rate during the first three stressful events showed an increasing trend, although not significant (p-value > 0.05), whereas in the fourth event, there was a significant increase compared to the other stress events, reaching average values of 110 bpm (p-value < 0.01). Regarding the recovery periods between stressful events, heart rate decreased but did not return to baseline values ($HR_{recovery}$ = 82-95 bpm; $HR_{baseline}$ = 63 bpm). Three hours after the test, heart rate still did not reach baseline values, showing values similar to those recorded during the first stress event ($HR_{final}$ = 78 bpm). During stressful moments, a significant increase in external acceleration was detected compared to the recovery periods (p-value < 0.001 in all cases).

These results demonstrate that heart rate and acceleration sensors are a key tool for monitoring welfare in aquaculture fish (Hvas et al., 2020; Palstra et al., 2024). During stressful situations, heart rate and acceleration increase, indicating that there is a higher oxygen demand associated with cardiovascular adjustments to maintain metabolism (Morgenroth et al., 2024). For this reason, it is essential to monitor oxygen concentration during typical stressful procedures in aquaculture.

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# ASSESSING INDIVIDUALITY OF EUROPEAN SEABASS *(Dicentrarchus labrax)*

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**Introduction**

Fish individuality is an understudied yet critical factor in aquaculture animal welfare, encompassing the consistent physiological and behavioural responses of individual fish to stressors. This individuality is typically classified into two distinct coping styles: proactive individuals, characterized by active, exploratory and bold behaviours, and reactive individuals, who are more docile, shy and evaluative in their responses. These coping styles have been associated to the regulation of gene expression, particularly involving genes related to cognitive processes and decision-making influencing, which may influence whether an individual adopts a reactive or proactive profile. This relationship between phenotype and genotype plays a key role in shaping how fish interact with their environment. Risk-taking behaviours, whether bold (proactive) or shy (reactive), is shaped by evolutionary pressures, as animals maximize fitness through behavioural plasticity and selection. Gene expression not only regulates these coping styles but also influences survival and reproductive success, especially under changing environments or stressing conditions, highlighting its importance for aquaculture. This study aims to investigate individual behavioural responses and cognitive abilities in European sea bass exposed to different challenges, identifying coping style patterns, explore the genetic basis of individuality, and discuss its implications for animal welfare management in aquaculture systems.

**Materials and Methods**

The experiment was conducted at the Marine Research and Aquaculture Laboratory (LIMIA-IRFAP) in Port Andratx (Mallorca). A total of 21 juvenile sea bass (7.81 ± 0.29 cm SL and 8.63 ± 0.43 g WW) were individually subjected to the *Cylinder task* test, a standardized protocol designed to assess inhibitory control functions in fish in response to an associated stimulus, by training individuals to obtain food from a plate placed inside a transparent cylinder. Following a 14-day acclimatization period, fish were individually transferred to aquaria (RAS system) comprising a "resting zone" with an enrichment element (artificial plant), and a "test zone". The experimental procedure spanned 7 test days per individual, structured into 4 phases: 1) Days 1 and 2: plate training. 2) Days 3 and 4: Cylinder familiarization. 3) Day 5: Forced door test using a cylinder. 4) Days 6 and 7: Cylinder + plate test. Video images of the different tests were recorded (5 minutes each test in phases 1, 3 and 4) and a range of behavioral parameters were quantified and analyzed with BORIS software. The behaviours recorded included swimming activity, exploratory behaviour, cylinder interaction, visual cue recognition, interaction with food or objects, surface activity, and stress-related behaviours such as immobility, thigmotaxis, and stereotypies. Upon completion of phase 4, fish were euthanized using anaesthetic overdose (5-10 ml/L) of 2-phenoxyethanol, followed by surgical removal of the telencephalon, which was used to quantify the expression levels of ten genes related to stress response, neuroplasticity, and behaviour (*mr, crf, crhbp, bdnf, npas4, dlg4, jph3, cfos, egr1, edrf1*) via qPCR, using *ef1a* and *β-actin* as reference genes for normalization.

**Results and Discussion**

Only 29% of individuals (n = 6) completed at least one phase of the experiment, and just one fish completed all stages. This low success rate likely reflects both the cognitive demands of the task and the species' sensitivity to acute stress. Nonetheless, PCA and heat map analyses based on behavioural metrics validated the Cylinder task as an effective tool for identifying coping styles in juvenile sea bass. Distinct behavioural profiles emerged, enabling classification into proactive and reactive types, with some individuals exhibiting intermediate or flexible traits. Notably, certain reactive fish displayed increased proactive behaviours over time, indicating adaptive flexibility. Gene expression patterns supported these behavioural trends (Fig. 1): reactive individuals overexpressed genes linked to stress regulation and neural plasticity (*npas4, crf, mr, edrf1, crhpb*), while proactive fish showed elevated expression of genes involved in exploration, neurogenesis, and memory (*bdnf, jph3, dlg4, egr1, cfos*). These results underscore the interplay between coping styles, gene expression, and behavioural individuality in sea bass.

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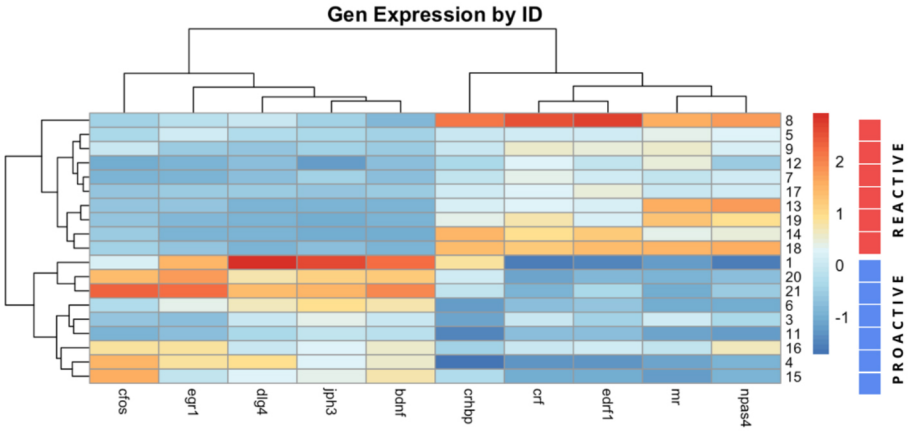


**Fig.1.** Scaled gene expression heat map (Z-score) of the 10 genes studied in *Dicentrarchus labrax*. Individuals were hierarchically grouped according to their gene expression profiles, revealing two main clusters associated with proactive and reactive coping styles. The genes represent key functions in stress regulation, synaptic plasticity, memory and exploratory behaviour.

**Acknowledgements**

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# EFFECTS OF FISH PEROXIDE TREATMENTS IN AQUAPONICS

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**Introduction**

Aquaponics integrates hydroponics (growing plants in a soilless medium) and fish culture in a controlled environment. However, culturing livestock and plants together presents ample opportunities for pests and diseases to invade the system, leading to crop yield reductions and monetary losses. Hydrogen Peroxide($H_2O_2$) has been used as an antibacterial and antiparasitic preventive treatment in aquaculture. However, it is unknown how fish peroxide treatments in aquaponics will affect the hydroponic culture, the biofilter, or the system.

**Material and methods**

Our goal in our experiment was to describe the effects of $H_2O_2$ treatments on aquaponics systems, specifically focusing on fish welfare, nitrogen balance, plant and root production, and water microbiome communities. To test this, we conducted 1-hour 200 ppm H2O2 treatments in a tilapia-basil aquaponic co-culture in a mid-size farm. After that, we measured the effect of the treatment on fish stress (cortisol excretion) and bacterial nitrification (health of the microbiome) over 21 days. Daily nitrification processes were tracked by measuring $NO_2$, $NO_3$, and $NH_4$ using a Hannah photometer. Fish welfare was monitored weekly by measuring water-borne cortisol at different points of the aquaponic system, as well as individual fish excretions of ammonia (NH4) and cortisol. We repeated this experiment three times over 3 months. At the end of each experimental period, we harvested the basil and recorded plant weight, height, leaf width, and root length. Moreover, we took water, root, and leaf samples for nutrient analysis.

**Results and discussion**

Our preliminary data showed peroxide treatments had a fast (48 hours) and long-term (21 days) effect. In the first two days after treatment, we found an increased concentration of ammonium by 2-3-fold. Additionally, the concentration of cortisol increased. These concentrations returned to normal values after a week. The increase of ammonia can be related to an increase in excretion of ammonium by fish after treatment, combined with peroxide damage to the biofilter denitrifying bacteria. At the end of the culture, we found that peroxide treatments significantly affected plant growth. However, this effect was not detectable to the naked eye, and the plants appeared healthy in both the control and treatment groups.

The results of our experiments indicate that, although it doesn't seem to affect plant production dramatically, the use of peroxide as a prophylactic or post-infection treatment must be monitored for possible nitrogen imbalances and damage to the microbiome in the biofilter.

# MODELLING LOW-TROPHIC AQUACULTURE CO-LOCATED WITHIN OFFSHORE WIND FARMS IN THE NORTH SEA UNDER CLIMATE CHANGE

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In line with the European Green Deal, the European Commission has adopted strategies and guidelines with ambitious visions for the offshore renewable energy sector and aquaculture sector. Aligned with this vision, the EU-funded ULTFARMS project aims to advance low trophic aquaculture systems, supporting their integration within offshore wind farms, while safeguarding the environment, minimising carbon footprint and addressing commercial viability. As part of the project, we modelled large-scale cultivation of sugar kelp and/or blue mussels, co-located within offshore wind farms in the North Sea, under climate change.

We used a 3D coupled hydrodynamic, water quality and ecological model of the Northwest European Shelf: the Dutch Continental Shelf model – Flexible Mesh (DCSM-FM). The DCSM-FM contains modules that simulate the growth dynamics of sugar kelp and blue mussels, including their interaction with the environment. The model also simulates the interaction of the wind turbines on ambient flow.

The DCSM-FM was nested in outputs from the global CMCC Earth System Model (CMCC-ESM2) for the SSP5-8.5 climate scenario. Aligning with the 2050 horizon of the European Green deal, we ran the simulations under the projected climate conditions around 2050. The climate change scenario, SSP5-8.5, is a scenario that was described by the IPCC. It is the scenario that arises from socio-economic trends described in the Shared Socio-economic Pathway 5, that lead to a radiative forcing of 8.5 $Wm^{-2}$ in 2100. Of the five SSP-based scenarios, SSP5-8.5 is the scenario with the highest amount of greenhouse gases.

The model provides insight into the effect of large-scale cultivation on the environment, such as changes in ambient nutrient availability and primary production. In addition, the model shows effects of wind turbines on current patterns, stratification regimes, fine sediment dynamics and any consequential changes in the growth dynamics of sugar kelp and blue mussels. Our findings help to identify the potential future impacts of large-scale seaweed and shellfish production on the North Sea ecosystem, and how climate change might alter the suitability of offshore wind farms for multi-use.

# CAN WE PREDICT NUTRIENT AND MINERAL DIGESTIBILITY OF PELLETED AQUAFEED DIETS BY *in-vitro* MODEL OF CULTIVATED SHRIMP GUT (*Litopenaeus vannamei*)?

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**Background**
Farming *L. vannamei* produced 11.96% of total aquaculture output in 2020. While easing pressure on wild stocks, sustainability depends on balanced, cost-effective feeds. Fish meal use has fallen from >25% in the 1990s to ≤12% in the past decade[1], replaced by plant, animal, fungal and bacterial ingredients. Plant sources offer cost and availability benefits but often contain indigestible carbohydrates, fibres and anti-nutritional factors[2]. Animal ingredients can also lack digestible nutrients. High saturated fat in animal-derived diets can impair growth, while PUFAs (EPA, DHA) are essential as shrimp cannot synthesise them efficiently; 0.86% dry weight is optimal. Fat requirements vary with salinity (6% at 40 ppt; 8% at 2 ppt). Meat/bone meal can replace 80% of dietary fish meal; blood/feather meals are limited to 30% without affecting growth[3]. Defatted insect meals can replace 60% of fish meal, but full-fat forms (high in saturated fats) are limited to 25%[4].

Phosphorus is vital for growth, but its digestibility varies. Liu et al. found phosphate digestibility of 52.1–79.9% and lipid digestibility of 2.1–92.5%[5]. Phosphorus-rich shrimp waste can trigger bacterial blooms and ammonia spikes, harming welfare and yield.

The *in vitro* salmon gut model "SalmoSim" has been used to evaluate novel feed ingredients. However, consultants in the aquaculture industry have been requesting a method to assess shrimp feeds, as feeding trials of cultivated shrimp are particularly arduous due to their small quantities of faecal matter. Here, we apply "ShrimpSim", an *in-vitro L. vannamei* gut model, alongside Pontus Research feeding trials to assess the digestibility of minerals and fatty acids in UK-produced feeds. Nutritional che

**Objective/ aim**
We aim to test ShrimpSim's ability to predict the in vivo digestibility of lipids, fatty acids and key minerals (Ca, P, Mg, Se, I, Zn, Fe) by measuring feed content before and after ShrimpSim assays on six diets.

**Methods**
Six InSuRAFeed pelleted diets (produced by Pontus Research with locally sourced Northern European ingredients) are being tested with ShrimpSim. Pellets are ground (mandible and gastric mill simulation), enzymatically digested and subjected to selective absorption (hepatopancreas simulation).

Nutritional chemical analysis will be conducted at University of Naples Federico II, thanks to the AQUASERV Transnational Access Programme. Absorbed and residual fractions will be lyophilised, homogenised and analysed via TQ-ICP-MS, Soxhlet and GC-MS for fatty acids and minerals. Digestibility coefficients will be calculated by comparing digesta with diets. Parallel *in vivo* trials at Pontus Research (Wales) will determine coefficients for crude protein, amino acids, lipids, fatty acids, Ca, P and energy.

**Expected Outcomes**
ShrimpSim is expected to screen ingredients for desirable mineral and fatty acid profiles. Regression analysis will compare ShrimpSim and *in vivo* results, with slope and intercept used to calibrate ShrimpSim predictions.

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**Significance**

Validating ShrimpSim for lipid fatty acid and mineral digestibility could provide the shrimp farming industry with a rapid, cost-effective tool to enable faster formulation of sustainable feeds. ShrimpSim has a rapid turnaround (1 week to generate digestates for up to four diets) compared to *in-vivo* trials (often 2-3 months). ShrimpSim supports fishmeal/ fish oil replacement, thus reducing the pressure on forage fish populations.

**Acknowledgments**

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# SHRIMP FARMING IN VIETNAM: NEW MODEL FOR EFFICIENCY, SUSTAINABILITY AND TECHNOLOGY TRANSFER

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Vietnam's shrimp industry is undergoing a period of profound stress, driven by persistently low farmgate prices, escalating input costs, and increasing scrutiny over environmental sustainability. These structural challenges are further intensified by global competition, particularly from cost-efficient producers such as Ecuador and India, resulting in a prolonged price crisis and declining profit margins for Vietnamese farmers.

In response, EcoSeafood Group (E.S.G) - a newly established enterprise building upon the technological foundation and field experience of ShrimpVet - has introduced a novel, systems-based shrimp farming model. This model integrates modular farm design, advanced production technologies, and mangrove agroecological integration to enhance productivity while minimizing environmental impact. With a current pilot site of 30 hectares, the system is engineered to optimize resource efficiency, reduce labor dependency, and lower carbon footprint through automation and scale.

E.S.G aims to expand this model to 50 modules by 2030, accounting for just 1% of Vietnam's total shrimp farming area, yet potentially matching national production levels through intensified yield. Beyond economic efficiency, the model addresses a broader socio-structural issue: attracting a new generation of farmers by offering a high-tech, profitable, and sustainable alternative to conventional shrimp aquaculture.

This presentation outlines the conceptual framework, preliminary outcomes, and long-term vision of this transformative approach to sustainable shrimp production in Vietnam.

# THE EFFECT OF SUPPLEMENTING DIET OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) WITH FISH MEAL AND CHEMICALLY PROTECTED SODIUM BUTYRATE ADDITIVE ON GROWTH AND RESISTANCE TO VIBRIOSIS

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**Introduction**

Feed efficiency and disease resistance represent the main economic challenges in aquaculture. For this reason, development of feeds, which improve growth but also reinforce the health fish status, is a priority for the sector. Different strategies can be implemented for this purpose, for instance a) inclusion of fish meal (FM) as source of protein to achieve better performance and b) supplementation with additives for the gut health benefits in livestock animals (Piazzon *et al*., 2017). European sea bass (*Dicentrarchus labrax*) (ESB) is one of the main farmed species in Mediterranean Basin and *Vibrio harveyi* one of the most concerning pathogens for this species, whose impact is also growing due to global warming (Amaro *et al*., 2020). This study investigates the effects of dietary incorporation of FM as well as supplementation with a chemically protected sodium butyrate (CpSB) additive on growth performance and vibriosis resistance in ESB.

**Materials and methods**

Functional feeds. Functional feeds were prepared by Spin-Off Life Bioencapsulation S.L. and the CpSB additive was provided by Novation 2000 SL. Diets contained 15 % (T15) or 30 % (T30) of fish meal (FM) and were supplemented with 0.2 or 0.4 % of the additive (T15 0.2, T15 0.4, T30 0.2 and T30 0.4).

Growth trial. Fish (Aquicultura Balear S.A.U, Palma de Mallorca, Spain) with 4.5g of average weight were kept in 250-liter tanks. with a 12-hour light/dark photoperiod, and fed *ad libitum* with a commercial diet along the acclimation period (1 month). Subsequently, a total of 960 fish were distributed in six experimental groups (n = 160 per group, with 4 replicates) and fed daily diets indicated above for 45 days. The control group received the commercial diet during the same period. Trial was performed in IATS fish facilities (ES1200330001055). Fish were weighed and measured before administering the different experimental diets and at the end of the feeding trial. Prior to handling, fish were anaesthetised with tricaine and euthanised with an overdose of the same anaesthetic.

Bacterial challenge. After the feeding period, an infection challenge with *V. harveyi* strain C2 was conducted by prolonged immersion (1 h) in seawater at 24± 2ºC, using the previously estimated lethal dose 50%. Within each group, 4 replicates of 20 fish were infected. After immersion, animals were transferred to 90-liter tanks. Moribund fishes were euthanized with tricaine, and internal organs were sampled on Trypticase Soy Agar with 1% of NaCl (TSA-1) to confirm *V. harveyi* as the cause of death (if the inoculated bacterium is recovered as a pure culture). Challenge ended when no mortality was recorded during, at least, 2 days, and survival rates were calculated.

Statistical analysis. Biometrical data were subjected to a one-way ANOVA. Survival rates were analysed using Kaplan-Meier method and Cox method to test for significant differences among diets, using R (version 4.5.1).

**Results and discussion**

All developed diets significantly improved fish weight and length, their impact on growth performance was therefore positive. Diets containing 30% FM yielded greater growth (11.5 g and 9.9 cm) than those with 15% (10.2 g and 9.6 cm), while additive supplementation alone had no clear effects (Fig. 1). However, in groups fed T30 diets supplemented with additive, a significant and dose-dependent increase in biometric parameters was observed (Fig. 1). This suggests that combining a higher level of FM with a high dose of the CpSB additive could enhance growth and be beneficial for sea bass farming.

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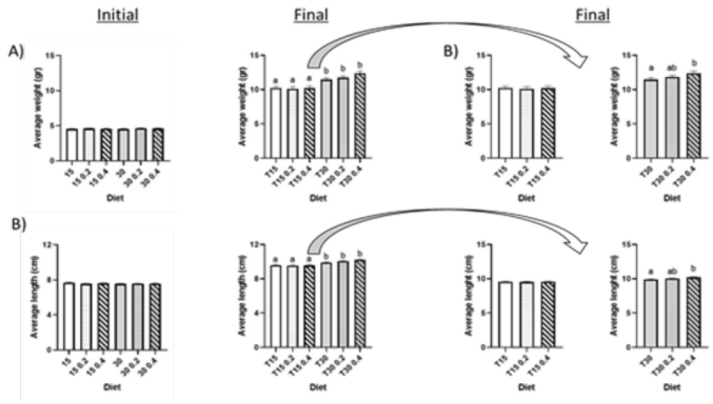


**Fig.1 Fish biometrical parameters. A)** Average weight and length of the fish (n=160) at the beginning and end of experiment. **B)** The average weight and length of the fish (n= 160) depending on the content of the fish meal diet (15 or 30%) at the end of the experiment. Data are expressed as means ± standard error of the mean (SEM). Different letters denote significant differences after one-way ANOVA (p<

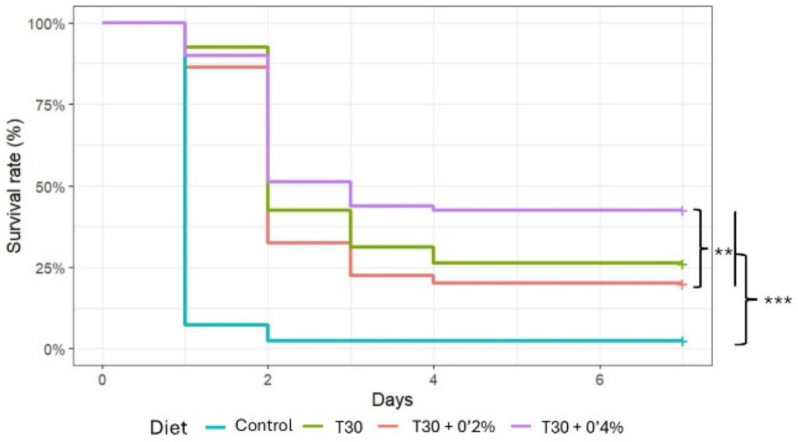


**Fig 2. Survival rates of sea bass groups fed different diets and challenged by immersion with *Vibrio harveyi***. Groups fed control diet, diet with 30% of fish meal (T30), diet T30 plus 0.2% or 0.4% of additive. Significant codes: p-value < 0.01 (**), p-value < 0.001 (***).

Fish fed T30 diets, the most promising ones in terms of growth performance, showed a significant increase in the survival rate after challenging with a high *V. harveyi* infection pressure (dose around $1x10^6$ cfu/mL), comparing with the control group (Fig. 2). Survival rate was significantly higher when T30 diet was supplemented with the greater dose (0.4%) of CpSB additive (Fig. 2).

## Conclusions

- Feeding with diets containing 30% of fish meal (*versus* 15%) over 45 days significantly enhances growth performance and survival of European sea bass (ESB) challenged with *Vibrio harveyi*.

- The inclusion of a chemically protected sodium butyrate additive at 0.4% in the diet leads to a notable increase in growth of ESB and improves their survival after *V. harveyi* challenge.

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## Acknowledgements

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# NORTH SEA SEAWEED EXTRACTS AS FUNCTIONAL AQUAFEED TO COMBAT AHPND IN *Litopenaeus vannamei* SHRIMP POST-LARVAE

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## Introduction

Despite their ecological impact, invasive seaweeds may offer a promising opportunity for sustainable aquafeed development. Along the North Sea coastline, the non-endemic green and brown seaweeds such as *Ulva australis* (southern sea lettuce) and *Sargassum muticum* (Japanese wireweed) are now widespread. Their inclusion in shrimp and fish diets could reduce reliance on costly and less sustainable ingredients like fish meal and fish oil. Additionally, many studies demonstrated the antimicrobial properties of seaweed extracts and components. This is particularly relevant in Pacific white-leg shrimp (*Litopenaeus vannamei*) hatcheries, where *Vibrio parahaemolyticus* infections cause acute hepatopancreatic necrosis disease (AHPND) and result in high mortality and economic losses. Vaccination is ineffective due to the shrimp's lack of adaptive immunity, and antibiotic use is strictly regulated to prevent the development of antibiotic resistance in the pathogen. Incorporating bioactive-rich seaweed extracts into pelletized feed may provide a functional immunostimulant solution that supports growth, disease resistance, and environmental sustainability. However, key questions remain about the specific bioactive compounds involved, optimal inclusion rates, and the cost-benefit analysis of the practical application in shrimp farming. This study aims to explore the potential of *U. australis* and *S. muticum* as alternative shrimp feed ingredients by evaluating their impact on shrimp growth performance and resistance to AHPND.

## Materials and Methods

Seaweed extracts from two *U. australis* strains and one *S. muticum* strain collected along the North Sea coastline were prepared using 70% ethanol and lyophilized. Bioactive compounds were analyzed via high-performance liquid chromatography (HPLC) and total phenolic content (TPC) assays. The minimum inhibitory concentration (MIC) against *V. parahaemolyticus* (AHPND strain M0904) was determined using microbroth dilution. A four-week feeding trial tested 1% and 3% inclusion rates of one of the seaweed extracts in shrimp post-larvae (PL) diets, assessing survival rate (SR), weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR). The control group was made of shrimp fed the standard diet without seaweed extracts. A pathogenic challenge evaluated the anti-*Vibrio* effects of these seaweed-supplemented feeds.

## Results

The MIC of both *U. australis* strains was 12.5 mg/mL, and that of *S. muticum* was 25 mg/mL. Classified as a phenolic acid and a flavonoid, respectively, gallic acid and quercetin were identified in all extracts and have been extensively studied previously as antimicrobial and antioxidant agents. The estimated TPC in *U. australis* strain NL, *U. australis* strain FR, and *S. muticum* extract was 269 ± 7.86, 69 ± 6.50, and 89 ± 8.65 µg gallic acid equivalent (GAE) per gram dried extract (Table 1).

Table 1. Phytochemical properties and antibacterial activity of seaweed extracts.

| Seaweed | Total Phenolic Content (µg GAE/g) | Minimum Inhibitory Concentration (MIC) (mg/mL) |
|---|---|---|
| *U. australis* strain NL | 269 ± 7.86 | 12.50 |
| *U. australis* strain FR | 69 ± 6.50 | 12.50 |
| *S. muticum* | 89 ± 8.65 | 25 |

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Preliminary results of the TPC and MIC values suggest that even low inclusion levels of seaweed extract in shrimp diets may confer protective effects against *V. parahaemolyticus*. A lower MIC combined with a higher TPC indicates stronger antimicrobial activity. Notably, experimental diets containing 3% of the *U. australis* strain NL showed promising trends in SR, WG, and SGR, with values of 97.78 ± 3.85%, 97.77 ± 26.16%, and 9.65 ± 1.97%/day, respectively, compared to 93.33 ± 5.77%, 92.70 ± 3.48%, and 9.37 ± 0.26%/day in the control group by week one. The FCR was considered relatively high concerning the digestive capacity in the adaptation phase in the first week of the experiment. Based on the SGR comparison, the treated PLs underwent efficient nutrient absorption from feed intake into their body mass. Key water qualities remained favorable and within the limits. These findings imply that seaweed supplementation has not had adverse effects and may contribute to enhanced immune function and growth performance in shrimp.

**Conclusion**

Selecting seaweed extracts with prospective antimicrobial and antioxidant potentials would reduce reliance on antibiotics or synthetic additives and aligning with sustainable aquaculture goals. Additionally, seaweed extracts may function as growth promoters to improve nutrient utilization. This study provides valuable insights for countries facing widespread macroalgae invasions and intensive aquaculture activities, where the challenges of managing foreign seaweed biomass, ensuring feed sustainability, and improving disease control must be simultaneously addressed. Understanding the relationship between the antimicrobial properties of invasive seaweeds as a dietary supplement and their therapeutic advantage on shrimp PL could be an ecologically and economically friendly prophylactic strategy to support stewardship in global aquaculture practices.

# AQUACULTURE AT EXPOSED SITES: CHALLENGES IN A NORTH SEA WIND FARM (EU-PROJECT OLAMUR)

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Many EU countries aim to be less dependent on global markets and create new income opportunities for coastal communities in a climate changing world. In addition to the already overused coastal areas of Germany, the site development plan for offshore wind farms in the German Bight will increase significantly over the next 25 years. Multi-Use (MU, Schupp et al. 2019) is intended to offer the possibility of joint use of the marine realm by different users without exerting additional ecological impact on the marine environment. Consequently, MU could pave the way for new stakeholders, such as offshore aquaculture enterprises, which will be conducted in synergy with offshore wind farms.

The OLAMUR-Project aims to assess the viability of MU aquaculture in three case studies representing differently exposed areas in the North and Baltic Sea. 70 km off the coast in Germany (case study A) low-trophic aquaculture experiments are located in the wind farm MeerWind Süd/Ost. After two years of planning, designing, testing of various system-designs, mooring concepts and farming strategies, farming of some of these selected taxa such as blue mussels (*Mytilus edulis*), European Oysters (*Ostrea edulis*), sea lettuce (*Ulva* sp.) and sugar kelp (*Saccharina latissima*) started in spring and early summer 2025.

Here, we present preliminary results (abiotic/biotic data, technology, moorings) of all farming trials. Results of this case study may provide a first insight whether aquaculture in very exposed offshore areas could be a potential new sector in the North Sea to achieve the above-mentioned aims.

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# ON-DEVICE AI-ENHANCED REAL-TIME FISH COUNTING SYSTEM IN AQUACULTURE

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**Introduction**
Aquaculture is a crucial industry in global food production, facing numerous challenges in efficiency, cost management, and environmental impact. Traditional methods of monitoring aquaculture rely on manual labor and primitive technologies, leading to inefficiencies and high operational costs. To address these challenges, this research focuses on developing an AI-empowered system capable of real-time fish counting underwater using on-device technology. By deploying advanced neural network models on edge devices, we aim to revolutionize aquaculture monitoring.

**Methods**
Accurate fish counting is essential for optimizing feeding strategies, which not only benefits fish farmers economically but also helps prevent marine pollution from excess feed. Key fish counting events include transferring juveniles into cages, monitoring growth phases in the cages, and during harvest. We have adopted different counting methodologies for each scenario: 1.Object Detection: Fish are often moved through specific checkpoints, making object detection suitable for counting during transfers. Correct camera positioning is crucial for this method. 2. Heat Map: In densely populated environments like entire cages, occlusion presents challenges for object detection; thus, a heat map approach is more appropriate.

We utilized a large-scale dataset consisting of videos collected on-site and synthetic videos generated via 3D CG simulations for training. The 3D CG generated data is especially valuable as it can be treated as ground truth. These datasets cover diverse environmental conditions, including varying lighting, water quality, and fish species. For the model training, we employed advanced deep learning techniques, we trained neural networks to achieve high precision and efficiency. For the edge deployment, the model is deployed onto chipsets near underwater cameras, enabling real-time processing and eliminating the need to transmit large volumes of video data.

**Implementation**
The system is designed to maximize efficiency and adaptability.Utilizing high-performance embedded chipsets like the Snapdragon 8 Gen 3 processor enables real-time video processing in embedded systems.Underwater cameras collect video data, which is processed locally on the embedded system. This reduces latency and communication overhead. Our application includes specialized neural network models for fish countings, and these models are all optimized for edge computing. Only essential metadata and summaries are sent to the central monitoring system, ensuring bandwidth efficiency.

**Results and Discussion**
We conducted fish counts during transfers and within entire cages. The deep learning models achieved 90%-95% accuracy in real-time underwater environments. However, for entire cage counts, visibility of the whole cage is crucial. Counting during transfers proved faster and less stressful for the fish compared to current mechanical systems. The on-device processing capabilities eliminated the need for extensive data transmission infrastructure, increasing system reliability and reducing overall costs.
These findings contribute to the following expectations. Real-time measurement of fish numbers and appetite indicators reduces feed waste and lowers production costs, enhancing feeding efficiency. Automated fish counting and size estimation during transfers increase process efficiency and operational productivity.

Future research will focus on several key areas. Enhancements to neural network models will continue to accommodate a broader range of environmental conditions and fish species. The system's scalability will be ensured for deployment in large, diverse aquaculture environments. Predictive analytics will be integrated to provide early warnings for potential issues like disease outbreaks and environmental risks. Intuitive user interfaces will be developed for easier adoption and operation by aquaculture farm operators.

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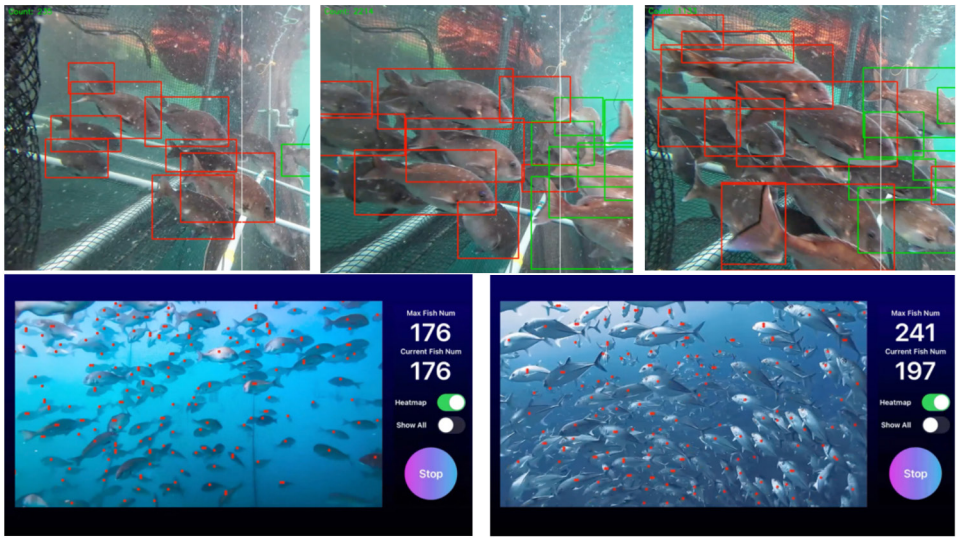


Fig. 1 Fish counting results: Above: by object detection (red seabreams); Bottom: a heat map method (left: red seabreams, right: tuna)

**Conclusion**

This research signifies a substantial advancement in aquaculture technology, providing a robust and efficient solution for farm monitoring. By leveraging advanced AI and edge computing, the study enhances operational efficiency, reduces costs, and improves the overall quality and safety of aquaculture practices. Anticipated future enhancements and expansions will further solidify this system as an indispensable tool in modern aquaculture.

# REAL-TIME FRESHNESS EVALUATION OF FISH USING AI-DRIVEN NEAR-INFRARED SPECTROSCOPY: APPLICATION FOR POST-HARVEST VALUE ENHANCEMENT

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**Introduction**

Post-harvest quality management is a critical issue in the global aquaculture industry. Fish prices are determined by species and weight, and the quality itself is often branded by origin by a few experts known as connoisseurs rather than by individual quality. By quantifying quality, it becomes possible to accurately reflect the value of fish in the market. Furthermore, in the supply chain of Japanese sea bream (Pagrus major), up to 15% of processed fillets are discarded annually due to uncertainty in freshness and subjective grading. This leads to a decrease in added value, increased food waste, and constraints on the predictability of shelf life. The K-value is a standard for evaluating the freshness of fish as per JAS (Japanes Agricultural Standards) regulations. Traditional K-value evaluation requires destructive, costly, and time-consuming laboratory HPLC analysis. To address emerging regulatory requirements and sustainability challenges (UN SDGs 12 and 14), we developed and validated a real-time, non-destructive freshness evaluation platform integrating handheld near-infrared spectroscopy (NIR) with advanced AI.

**Materials and Methods**

Sampling and Workflow: We sampled 1,178 skin-on fillets of [Pagrus major] farmed in the Sea of Japan over 0-7 days post-mortem, reflecting distinctive post-harvest conditions in Japan. The fillets were scanned at three standardized positions using two InnoSpectra MEMS NIR devices (900–1700 nm and 1350–2500 nm, 3 mm spot, 2-second acquisition). The scanning was implemented by workers using mobile or tablet interfaces during the packaging process.

Reference Method: The K-value ([HxR+Hx]/[ATP+ADP+AMP+IMP+HxR+Hx]×100) was measured by two inspection agencies, which measured samples on the day of slaughter and three days later. The two agencies measured the same samples simultaneously to confirm consistency in results.

Spectral Processing and Modeling: Each spectrum was processed using a triangular filter bank and differential feature extraction, then normalized (mean 0/variance 1, training set). Model Pipeline: RandomForestRegressor (scikit-learn), hyperparameters optimized with nested CV. An 80/20 split was used for training/testing sets (n=942/236), with 10-fold cross-validation conducted. Outliers (≤2%) were detected using Mahalanobis distance and excluded from model training. Cloud integration supported immediate result transfer and batch traceability.

**Results**

The NIR-AI platform achieved an MAE=1.62, RMSE=2.46, and $R^2$=0.74 for K-value estimation in the test set, attaining a 91% category accuracy (Sashimi: K<20%, Cook: 20-40%, Reject: >40%), outperforming both baseline statistics and subjective evaluations. The shelf-life prediction curve using the NIR system matched observed spoilage and sensory cut-off points (average $\Delta t$ <1 day). The real-time analysis results from the NIR sensor showed a high accuracy, with the Lab test K-value at 15 compared to our model's analysis of 15.1.

**Discussion and Impact**

Comparison with State-of-the-Art: Unlike traditional linear regression or lab-supported NIR solutions, our deep learning and SaaS-connected approach enables real-time, operator-independent automation. Each test costs less than €0.002 (compared to €250 for HPLC) and can handle TEU-level processing capacity with five handsets per line.

Traceability and Scalability: The cloud-integrated results enable dynamic FIFO stacks, price optimization, and regulatory audit trails, essential for achieving EU Green Deal/"Farm to Fork" goals.

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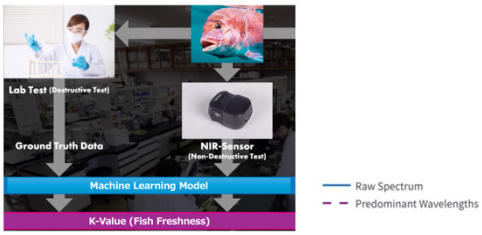


Fig. 1 Overview of our system

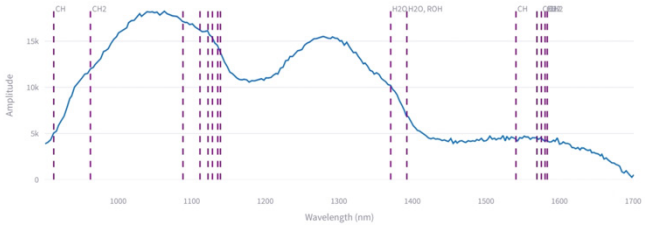


Fig.2 An example of NIR sensor data of a red seabream. It shows the explainability.

**Deployment and Future Work**

The current model is developed for sea bream, with plans to expand to other fish species. Pilot installations are scheduled for Mediterranean mackerel and bluefin tuna lines. Future upgrades will include outputs for microbial spoilage and nutritional components, as well as blockchain or digital twin features for farm-to-fork traceability. We are actively seeking collaboration with additional processing plants in the EU, NGOs, and retailers.

**Conclusion**

This study validated a real-time AI/NIR platform for fillet grading that directly addresses post-harvest value, waste, labor efficiency, and Green Deal SDG goals. This system establishes a new digital standard for aquaculture quality management in the EU and Japan.

**Acknowledgements**

We thank Akasaka Fishery, Ehime Institute of Industrial Technology, Merieux Nutrisceince Japan Corp. (formerly Bureau Veritas FEAC), InnoSpectra, Balfegó, and our technical, academic, and industrial partners. Part of this study was funded by the Triangle Ehime Promotion Project's "Digital Implementation Acceleration Project".

# DIETARY EFFECT OF *Bacillus subtropicus*_W1 SP. NOV. ON *Macrobrachium rosenbergii* AS A POTENTIAL PROBIOTIC CANDIDATE

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**Introduction:**
The application of probiotics in the aquaculture industry of Bangladesh has been gradually increasing in recent years; however, the probiotic industry of the country, worth $410 million, is solely dependent on imported products. Country of origin, transportation and storage conditions of these imported probiotics vastly affect the performance compared to the price (Wardad, 2024). Spore-forming bacteria, mostly of the genus *Bacillus,* exhibit probiotic properties (Elshaghabee et al., 2017). Several *in vitro* and *in vivo* models revealed that members of this genus comply with the standards for being probiotic candidates, have tolerance to acidic substances and are competent to thermal stability during processing and storage conditions (Bader et al., 2012). To reduce dependency on imported probiotics, this study aimed to isolate probiotic candidates from the indigenous sources, i.e., river and canal water. Bacterial identification was done by whole-genome analysis, and probiotic properties were evaluated by several *in vitro* and *in vivo* assays.

**Material and methods:**

Bacteria were collected from the Bishna River and Talbuniya Lake in Bagerhat, southern Bangladesh, and plated onto HiChrome Bacillus Agar for genomic DNA extraction. Library preparation was performed using the Rapid Barcoding Kit 96 V14 (Oxford Nanopore Technologies, Oxford, UK). Long read DNA sequencing was conducted using PromethION R9.4.1 flow cell chemistry. Assembly and annotation were performed using Flye (2.9.6) and the Bacterial and Viral Bioinformatics Resource Centre (BV-BRC), respectively. Type Genome Server (TYGS) was used to construct the phylogenetic tree. The endurance test of the bacteria was performed following the method previously described by (Gao et al., 2024) with a slight modification. Efficacy of the locally isolated strains Bacillus subtropicus_W1 sp. Nov. (W1), Rummeliibacillus bengalensis_S3 sp. Nov. (S3), B. pacificus_S5 (S5) and Priestia bengalensis_S6 sp. Nov. (S6) were evaluated both individual and in combination, in parallel with a commercial probiotic in a feeding trial with a control (commercial fed with no probiotic added) in triplicate. In the feeding trial, commercial feed coated with bacterial culture (~103 cfu/ml) was fed twice (5% of body weight) daily for 5 weeks. Average weight, total haemocyte count (THC), pro-phenoloxidase (Pro-PO) and digestive enzyme activity (amylase, lipase and protease) were evaluated to justify the probiotic potential.

**Results:**
The assembled genome of the novel isolate *Bacillus subtropicus*_W1 possessed 2 contigs, with a total length of 5,572,840 bp (Contig1:236880bp, Contig 2: 5335960bp) and an average G+C content of 35.38%. This genome consisted of 5,728 protein-coding sequences (CDS), 106 transfer RNA (tRNA) genes, 14 complete operons and an extra 5S gene. The annotation included 1,442 hypothetical proteins and 4,286 proteins with functional assignments (Figure 1A). The phylogenetic tree placed the isolate next to *Bacillus tropicus* with dDDH value < 70% (Figure 1B). The isolate showed survival potential at extremely high temperatures (60°-100°C), acidic (pH 2) and basic (pH 10) conditions. Prawns fed the *B. subtropicus*_W1 coated diet resulted in higher ($p<0.05$) average weight, total haemocyte count, Pro-PO, amylase, lipase and protease enzyme activity than those fed the control diet and commercial probiotic supplementation. In contrast, haemocyte clotting time was significantly lower in the group fed *B. subtropicus*_W1-coated diet, indicating better health condition (Figure 2).

**Conclusion:**
Findings of the study showed that locally isolated *Bacillus subtropicus*_W1 sp. Nov. possesses great potential as a probiotic candidate for the aquaculture industry of Bangladesh along with the countries experiencing temperature rise due to global warming.

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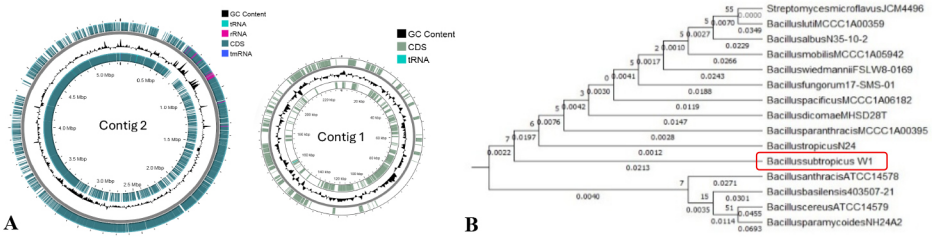


**Figure 1.** (A) Genome organisation of *Bacillus subtropicus*_W1 sp. nov. showing GC content, predicted coding sequences (CDSs), transfer RNAs (tRNAs), and ribosomal RNAs (rRNAs). (B) Phylogenetic position of *Bacillus subtropicus*_w1 sp. nov., inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences.

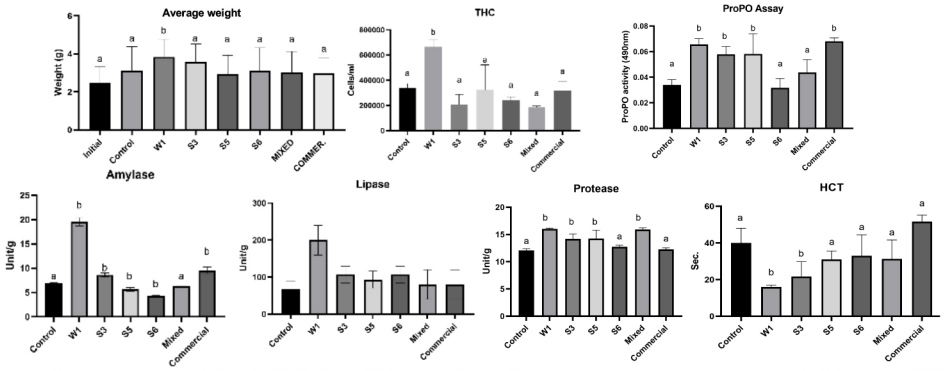


**Figure 2.** Average weight, THC, Pro-PO, amylase, lipase, protease enzyme activities and HCT in prawns fed with different bacterial-supplemented diets.

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# FISH FARM PRODUCTIVITY AND THE VALUE OF "SUPER-SITES" IN SALMON FARMING IN NORWAY

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**The value of "Super-sites"**

Salmon farmers in Norway talk about "super-sites", for sites that are especially suited for grow-out of salmon, and which have especially high productivity. High productivity can however be measured in different ways, and there may be different types of qualities and effects that can make an area a "super-site". The productivity can e.g. be measured in terms of the total average volume produced to slaughter per year, the effective economic feed factor, or in pecuniary terms (low production costs or high profitability).

Factors that describe and explain competitiveness between salmon producing nations include growth rates, feed conversion ratio, harvest weight, smolt yield and mortality for the farmed fish (Iversen, Asche, Hermansen, & Nystøyl, 2020). The same factors may explain what makes a site a super-site. In addition, the prevalence of lice, disease and external shocks like algae blooms and string jellyfish may strongly affect productivity and profitability.

While some literature find that biophysical factors does not explain profitability (Zhang, Sogn-Grundvåg, He, Tveteras, & Xu, 2025), this is probably because they fail to recognize the great variation among sites. Measuring productivity at firm level (some firms holding up to 300 sites) you effectively cancel out the variation between sites in terms of temperature, growth, lice prevalence, mortality et.c. Analyzing productivity at the site-level (using data on pen level to improve data on growth and feed conversion ratios) will give a significant contribution to the literature on biophysical factors and productivity.

Under the newly proposed production regulation regime in Norway, the productivity of aquaculture sites will be of increased importance, as the productivity of the site will be a strong determinant of company value.

**This presentation**

We will present an analysis that identify actual super-sites in Norwegian grow-out salmon farming. These will be based on different criteria and criteria-sets for what defines a super-site. The simplest measure is its revealed capacity to produce over time.

The production capacity of the site as defined by the aquaculture license (which is in terms of the maximum biomass in tonnes that can be held at the site at any given time), but real production capacity varies much more than licensed capacity, dependent on depth, currents, how long fallowing periods are needed between generations to restore sea bottom conditions, average lice numbers on the salmon in the farm and the need for lice treatments to stay within the mandatory limits, disease outbreakset.c. Revealed capacity is illustrated in figure 1 (each site on the x-axis). This shows the combined production for each site over the last ten years. Production ranges from close to 45.000 tonnes, or 9000 tonnes over each two-year production cycle. Only 1/3 of the sites have shown a production of more than 10.000 tonnes for these ten years.

The analysis will describe the distribution of growth rates, mortality, feed conversion ratio, smolt yield et.c., and analyze how these parameters are explained by temperature, lice prevalence et.c. Even for these factors variation is huge, as exemplified by mortality in figure 2. Mortality ranges from over 50 % down to 1-2 % for the best sites.

The analysis will also include how different operational characteristics and parameters for individual farms as well as groups of adjacent farms may help explain the variation in performance and what makes a site a super-locality. The analysis is based on a dataset with production data from all individual pens in Norway for the period 2005-2024. This is combined with data on lice prevalence, lice treatments and disease. The huge range of productivity between sites will be translated into economic effect by a techno-economic model as used in (Holan et al., 2017; Iversen, Hermansen, Nystøyl, & Hess, 2017).

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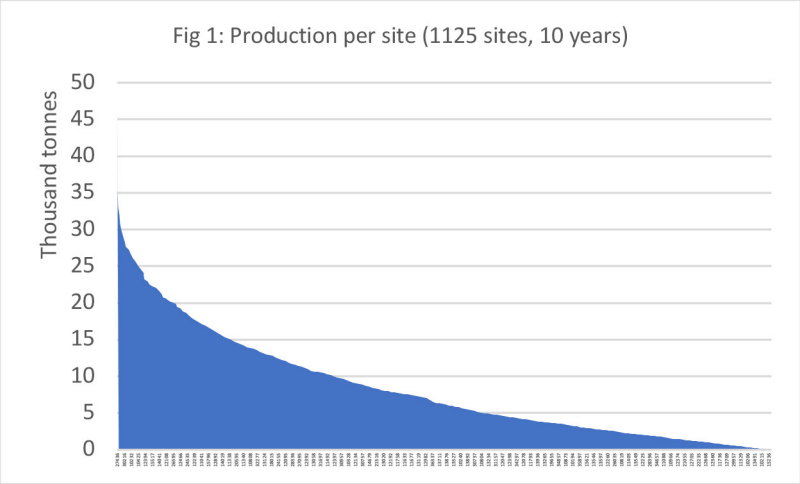
Fig 1: Production per site (1125 sites, 10 years)
Thousand tonnes
50
45
40
35
30
25
20
15
10
5
0

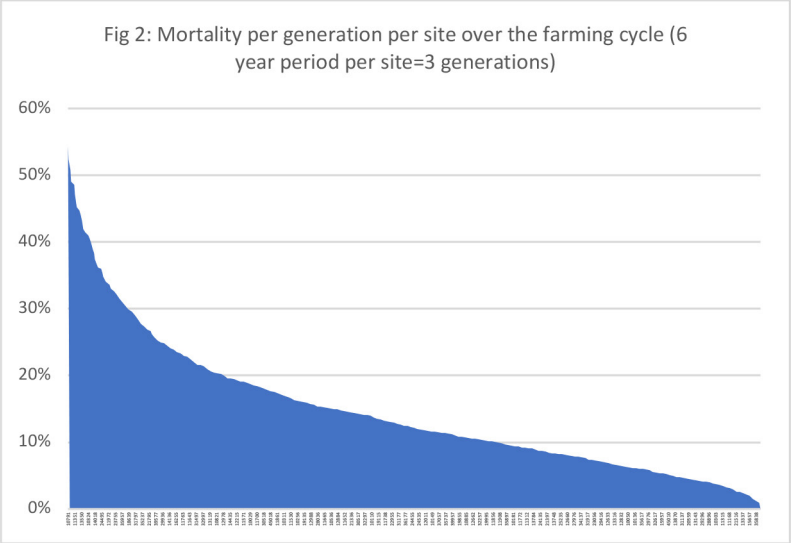
Fig 2: Mortality per generation per site over the farming cycle (6 year period per site=3 generations)
60%
50%
40%
30%
20%
10%
0%

# EXPLORING *Punica granatum* LEAF-DERIVED POLYPHENOLS AS INNOVATIVE PREBIOTICS FOR SYNBIOTIC FORMULATIONS IN SUSTAINABLE AQUACULTURE

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**Objectives:**
Pomegranate (*Punica granatum* L.) leaves, rich in bioactive polyphenols, are promising agri-food byproducts for sustainable health applications. Despite their known antioxidant and antimicrobial properties, their use in synbiotic systems with probiotic yeasts remains underexplored. This study evaluates the prebiotic effects of pomegranate leaf extracts on *Saccharomyces boulardii* and their antimicrobial activity against fish pathogens, highlighting their synbiotic potential for sustainable aquaculture.

**Methods:**
Pomegranate leaf extracts were obtained via microwave-assisted hydroethanolic extraction and analyzed for chemical composition, antioxidant capacity, and antimicrobial activity against aquaculture pathogens. Their prebiotic effects on *S. boulardii* were assessed through a fitness assay at varying concentrations. Extracellular and intracellular postbiotic fractions were isolated and evaluated for polyphenol content, antioxidant activity, soluble protein levels, and antimicrobial properties. Extracellular postbiotics were recovered from the cell-free supernatants, while intracellular compounds were extracted via cell lysis and subjected to the same analytical assessments.

**Results:**
The hydroethanolic extract of pomegranate leaves exhibited high total polyphenol content, strong antioxidant capacity, and notable antimicrobial activity against aquaculture pathogens. Additionally, it significantly enhanced the growth of *S. boulardii* in glucose-free medium over a 7-day incubation, demonstrating strong prebiotic potential. Pomegranate leaf extract enhanced extracellular and intracellular postbiotic fractions, increasing polyphenols, antioxidant activity, and proteins, with extracellular postbiotics showing the highest bioactivity. Antimicrobial assays demonstrated that pomegranate leaf extract and postbiotic compounds of *S. boulardii* exerted inhibitory activity against *Aeromonas veronii* and *Aeromonas hydrophila*, two significant pathogens associated with infectious diseases in aquaculture systems.

**Conclusions:**
Pomegranate leaf extract enhances *S. boulardii* growth and postbiotic activity while showing antimicrobial effects against aquaculture pathogens. This highlights the combined potential of both *S. boulardii* and pomegranate leaf extract as sustainable synbiotic ingredients to improve fish health and reduce antibiotic use in aquaculture.

# BACTERIAL CONTAMINATION IN SHELLFISH – ENDOTOXIN PROVIDES A RAPID TESTING SOLUTION

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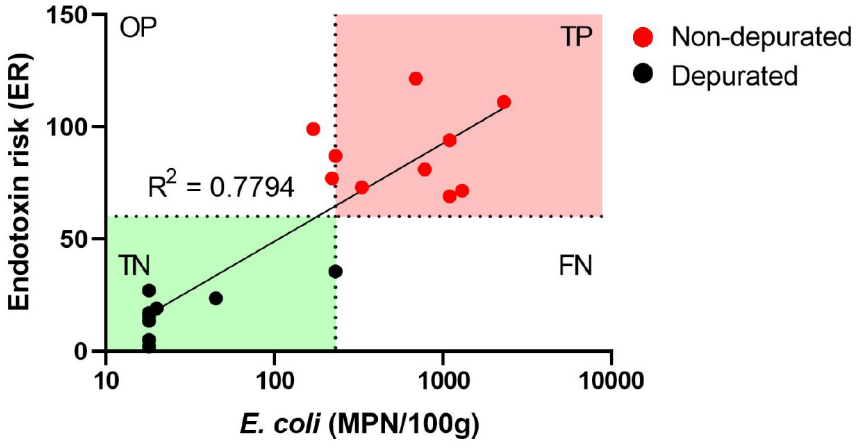
**Background**

Shellfish are filter feeders that can accumulate harmful pathogens, including bacteria (e.g., *Vibrio* spp, *Salmonella* spp, *Shigella* spp, *Campylobacter* spp and pathogenic *Escherichia coli*). Culture of *E. coli* in shellfish still represents the primary way of assessing the microbiological quality of shellfish. The reference methods (EN/ISO 6888-1 and 2 of 1999) require incubation periods ranging from 24 to 48 hours (or longer), to allow bacterial colonies to grow, which delay results and potential public health protection actions. This presents several problems including the long time to results and focus on *E. coli*. Moreover, culturing bacteria requires trained personnel, specialized lab facilities, and expensive reagents. These factors make it less practical for high-throughput or field-based testing, especially in resource-limited settings.

Shellfish classification categories are based on permitted levels of E. coli/100g flesh and intravalvular liquid. The disadvantages of culture-based testing for bacterial contamination highlights the need for new rapid, sensitive, field-based tests to detect bacterial contamination of shellfish to allow proactive measures to protect public health.We have previously described a validated method to rapidly assess bacterial contamination in recreational water (Good et al., 2024; Sattar et al., 2022). This is based on the detection of a bacterial molecule (endotoxin). In this study, we describe an adaptation of this method to rapidly detect Gram-negative bacterial contamination of shellfish that can be used in the field by non-technical staff. This study has established the feasibility of a novel method to rapidly detect bacterial contamination in oyster flesh and has shown that the Endotoxin Risk (ER) value significantly correlates with *E. coli* content in oysters.

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**Methods**

Two hundred pacific oysters (Crassostrea Gigas) were sourced locally in Devon; 100 of the oysters had been depurated and 100 had not been depurated. The oysters were stored at 4°C ± 2°C and were examined within 48 hours. The amount of *Escherichia coli* in the oysters was enumerated by the Most Probable Number (MPN) method, following the ISO 16649-1. Endotoxin was measured using the Bacterisk assay (Molendotech Ltd., Brixham, UK; www.molendotech.com) and was performed following the manufacturer's instructions. The Bacterisk device was pre-calibrated using known endotoxin standards (0.1-10 EU/mL) and against known reference concentrations of E. coli (10-5000CFU/100mL) Output from the Bacterisk device is given in Endotoxin Risk units (ERU) which are calibrated from known endotoxin standards. The ERU values can be equated with known concentrations of *E. coli* (CFU/100mL) to give threshold and cut-off values (Good et al., 2024)

**Results**

By using the threshold for class A shellfish of 230 *E. coli*/100g and setting a proposed equivalent threshold of 60 ERU it was possible to split the data into quadrants (see Figure 1) to show off-target positives (i.e. detection of other Gram-negative bacteria) (n=3), true positives (n=7), true negatives (n=10) and false negatives (n=0). From this the following was calculated: Sensitivity: 100%, Specificity: 77%, False negative rate: 0% and Off-target[1] positive rate: 30% The specificity and off-target rate reflect that this test will also respond to bacteria other than *E. coli* including pathogens such as Salmonella spp and Vibrio spp and can thus alert to the contamination of shellfish with pathogens not detected by current methods.

**Conclusion**

This study has established the feasibility of a novel method to rapidly detect bacterial contamination in oyster flesh and has shown that the Endotoxin Risk (ERU) value significantly correlates with *E. coli* content in oysters. As microbial threats evolve, continued investment in rapid detection technologies and regulatory oversight will be essential for maintaining public health standards.

# EFFECT OF FISHMEAL QUALITY ON DIGESTIBILITY, GROWTH PERFORMANCE AND FEED UTILIZATION IN ATLANTIC SALMON (*Salmo salar*)

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## Introduction

Fishmeal represent a major component of aquafeeds due to its high protein quality, balanced essential amino acid profile, high digestibility and palatability, essential traits for feed efficiency and optimal growth of farmed fish, such as Atlantic salmon (*Salmo salar*) (Hasan et al., 2023). However, variability in fishmeal quality, different species of fish, raw material freshness, processing conditions, and chemical composition, can influence the nutritional value of fishmeal (Mundheim et al., 2004). The purpose of this study was to assess the effect of fishmeal quality on digestibility and growth performance in Atlantic salmon (*Salmo salar*) raised in RAS, as part of a larger effort to profile and benchmark the majority of commercially available fishmeal products in the Icelandic market; with an ultimate goal to assist with a calibration predictive model using near-infrared (NIR), which allows rapide and accurate determination of fishmeal quality and its nutrient potential.

## Materiel and methods

Atlantic salmon (initial mean weights of 121g) were fed experimental diets formulated based on the protocol developed by Cho et al., (1982) where a reference diet of high palatability will be formulated with a known digestibility and a test diet in a ratio of 70:30 (70% reference diet and 30% of test ingredient), where only the fishmeal source is different. Seven fish meals (Sandeel (*Ammodytes* sp) 73%, Sandeel (*Ammodytes* sp) 55%, European anchovy (*Engraulis encrasicolus*) 40%, Gulf menhaden (*Brevoortia patronus*), Blue herring (*Clupea harengus*), Mackerel (*Scomber* sp) trimmings 1, Mackerel (*Scomber* sp) trimmings 2) and one reference commercial fish meal were tested (in triplicate tanks = 24 tanks). All fish were kept under stable water conditions and overfed over six weeks. Daily, uneaten feed was removed to calculate feed intake. At the end of the trial, all fish were weighed individually, and feces were collected by stripping to determine the Apparent Digestibility Coefficients (ADC) of crude protein and amino acids using yttrium oxide as an inert marker.

## Results

Growth performance indicators were positively correlated with crude protein and essential amino acid digestibility. Strong negative correlations were observed between ash and NaCl, and digestibility. Crude protein digestibility and methionine digestibility were identified as primary drivers for variation among fishmeal for growth rate and feed conversion ratio (FCR). Predictive models indicated that digestibility values could explain up to 83% of the variability in Specific Growth Rate (SGR). Quality indicators (TVBN and biogenic amines; histamine, putrescine, and tyramine) had negative associations with nutrient digestibility indicating an indirect effect on growth due to reduced bioavailability. Notably, putrescine concentration, a major spoilage marker, was linked with lower digestibility and poorer growth. Among the products tested, a fishmeal produced from mackerel trimmings showed the best performance in growth and feed efficiency, while a European anchovy meal had the lowest performance.

## Conclusion

Fishmeal quality plays a significant role in nutrient digestibility and growth performance of Atlantic salmon. Poor digestibility and growth efficiency were associated with high levels of spoilage indicators, like total volatile basic nitrogen (TVBN), histamine, putrescine, and cadaverine. The current data highlight the importance of raw material freshness and processing quality in determining fishmeal nutritional value. For optimized feed utilization, improved growth performance, and enhanced sustainability in aquaculture, high crude protein digestibility and low spoilage is required in fishmeal.

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# ENHANCED FISH WEIGHT ESTIMATION THROUGH YOLO AND ELECTRODE DATA INTEGRATION

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## Introduction

Accurate fish weight estimation is critical to efficient aquaculture management, impacting feeding optimization, health monitoring, and harvest scheduling. Non-intrusive methods have gained significant attention for their ability to streamline this process without inducing stress in fish populations (Li et al., 2020). Among these methods, the You Only Look Once (YOLO) object detection framework has emerged as one of the most widely used approaches for non-intrusive fish weight estimation due to its high accuracy in detecting and analyzing fish morphology from images. However, YOLO's performance can be limited when dealing with fish sizes and conditions not represented in its training data, particularly in extrapolation scenarios (Tengtrairat et al., 2022).

## Method

To address these limitations, we propose an enhanced method integrating electrode-based voltage measurements with YOLO-driven image processing, leveraging machine learning (ML) regression models for fish weight estimation. While electrode measurements have traditionally been utilized for applications such as fish counting, their integration with visual data for weight estimation remains underexplored (Sheppard & Bednarski 2015). By combining these two complementary data sources, our approach aims to improve predictive accuracy, especially in challenging conditions where image-only models struggle, with affordable component prices.

Our model was evaluated under two distinct conditions: interpolation, where YOLO performed well due to familiar weight ranges in the training data, and extrapolation, where YOLO's detection accuracy was reduced due to unfamiliar fish sizes. The integrated model consistently outperformed image-only models in both case studies, with statistical significance. This outcome demonstrates the robustness of the combined approach, as electrode measurements provide additional physiological insights that compensate for the limitations of image-based models.

## Results

The results underscore the potential of integrating multi-modal data sources to enhance non-intrusive fish weight estimation, offering cheap and a more resilient and accurate solution for aquaculture operations.

| Method | YOLO MSE [$gr^2$] | Model MSE [$gr^2$] |
|---|---|---|
| Interpolation | 2.82 | 1.74 |
| Extrapolation | 6 | 4.3 |

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# CO CULTURE OF *Palmaria palmata* WITH FINFISH ON LAND, OPPORTUNITIES IN NORWAY

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*Palmaria palmata*, commonly known as ‘dulse’ or ‘søl’ in Norwegian is a well know red seaweed that has relatively high value as a human food product. There is considerable interest in growing the species throughout Norway, Europe and other countries worldwide, and Nofima has been investigating the specific opportunities in the north of Norway.

Research at Nofima has focused initially on protocols for maximizing the spore release and utilization from seaweed collected in the north of Norway. Optimal protocols have now been defined for P. palmata in the north of Norway and work is continuing to develop techniques for commercial seeding onto ropes.

Novel techniques and growing systems for growing *P. palmata* have also been explored. These have focused on growing *P. palmata* in discharge water from land-based finfish facilities in the north of Norway. Seawater was monitored and collected from a large land-based RAS salmon smolt facility on the island of Kvaløya in northern Norway. A series of trials were conducted to test the capacity of *P. palmata* to grow in discharged water from these facilities. These included trials at various inclusion rates of discharge water to find the optimal growing conditions. The results showed that approximately 50% inclusion of discharged seawater was optimal. Further trials showed the influence of varying salinities on the growth of *P. palmata*. The water analysis during these trials also allowed the calculation of production capacity figures for *P. palmata* based on discharge rates from land-based RAS facilities. This presentation presents the results of these trials and discusses the potential to commercialize such activities in the north or elsewhere in Norway.

# GROWTH PERFORMANCE, FILLET YIELD AND SOMATIC INDICES OF EUROPEAN PERCH (*Perca fluviatilis*) FED DIETS CONTAINING VARYING LEVELS OF *Ganoderma spp*. COMPOSITE MYCELIUM MEAL

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**Introduction**

In aquaculture, maximizing growth performance and fillet yield while maintaining good fish health is crucial for sustainable and economically viable production. With increasing pressure to replace conventional feed ingredients such as fishmeal and soybean, novel and functional alternatives are gaining traction. Composite mycelium meal (CMM), derived from *Ganoderma spp*. through solid-state fermentation (SSF) of agrifood side-streams, offers promising nutritional and bioactive properties. While the potential of *Ganoderma*-based ingredients has been explored in various species, evidence for its effects in European perch (*Perca fluviatilis*) is lacking. This study investigates how different inclusion levels of CMM affect growth, fillet yield, and somatic indices in European perch.

**Materials and methods**

Juvenile European perch were fed one of four diets for 12 weeks: CTRL (0% CMM), CMM-5 (5% CMM), CMM-7.5 (7.5% CMM), CMM-10 (10% CMM).

The CMM was co-developed by KIDEMIS and BFH-HAFL using *Ganoderma spp*. through SSF. At the end of the trial, fish were measured and dissected to evaluate final weight, fillet yield, hepatosomatic and visceral fat index.

Data were analyzed using linear models in R (lm function), with estimated marginal means calculated using the emmeans package.

**Results**

Growth performance:
Fish in the CMM-10 group had significantly higher final body weight compared to the CTRL and CMM-5 groups ($p < 0.05$).

Fillet yield:
Despite differences in body weight, fillet yield did not significantly differ between dietary groups.

Somatic indices:
Hepatosomatic index showed a progressive but non-significant increase with higher CMM levels. However, visceral fat index was significantly higher in the CMM-10 group compared to CMM-7.5.

**Conclusion**

Composite mycelium meal derived from *Ganoderma spp*. supported enhanced growth in European perch, particularly at the 10% inclusion level. However, the increase in visceral fat and organ weight without a corresponding rise in fillet yield highlights potential shifts in energy partitioning. These metabolic effects require further investigation to determine long-term implications and optimal inclusion levels. The results indicate that while CMM is a promising sustainable ingredient, moderate inclusion rates may offer a more balanced outcome for performance and product quality.

# ARTIFICIAL INTELLIGENCE (AI) TOOLS USED IN AQUACULTURE FACILITIES IN THE EUROPEAN UNION, WITH RESPECTIVE COUNTRIES

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Aquaculture has become a vital component of the European Union's food production system, contributing significantly to the economy and food security. Due to the increased demand for fish, a quest for novel and efficient techniques in aquaculture production, systems to alleviate the burdens of fish disease management, environmental degradation, and high production costs have become imperative. The integration of Artificial Intelligence (AI) tools in aquaculture facilities across the European Union (EU) has emerged as a promising solution for rapidly transforming and enhancing productivity, sustainability, operational efficiency, and competitiveness. Prominent corporations and academic institutions have created innovative artificial intelligence (AI) solutions, including autonomous underwater vehicles (AUVs) for monitoring and maintenance in offshore environments, real-time behavioral monitoring systems for accurate feed dispensing, and AI-powered cameras for fish size and growth analysis.

AI-enabled sensors, as demonstrated in EU-funded projects like ASTRAL, provide continuous, automated water quality monitoring, utilizing machine learning algorithms to detect anomalies and environmental risks, thereby promoting sustainable and high-quality production. Across the EU, AI tools are being integrated into aquaculture to address key operational challenges. Countries like Norway and France use smart cameras and feeding systems for biomass estimation and waste reduction, while Denmark and Italy apply AI for real-time water quality and disease monitoring. Spain, Greece, and the Netherlands leverage AI for health grading, production forecasting, and biofouling control, showcasing a shift toward precision and efficiency in aquaculture. Table 1 shows the AI tools currently used in aquaculture facilities across the European Union.

By harnessing AI, EU aquaculture is transitioning toward data-driven, resilient, and eco-efficient production systems. These innovations reduce labor costs and environmental impact and improve productivity and animal welfare. The integration of AI in European aquaculture aligns with policy objectives such as the European Green Deal, and the Farm to Fork Strategy by providing a path to overcoming the economic, environmental, and social burdens that have long challenged the sector.

**Table 1.** Key AI Tools in EU Aquaculture

| Tool/Project Name | Country/Region | Main Applications |
|---|---|---|
| AquaREAL | France, EU-wide | Predicting water quality, optimizing fish welfare |
| EdgeSpot Sensor | EU-wide (ASTRAL) | water quality monitoring, anomaly detection |
| iFarm | Norway, EU | Individual fish identification, health monitoring |
| Aquaculture 4.0 | Spain | Biomass analysis, fish welfare monitoring, and decision support |
| Fish-AI | Italy, Belgium, Portugal | Artificial intestine for predictive feed assessment |

# IMPROVING THE NURSERY CULTURE OF THE GREENSHELL™ MUSSEL IN NEW ZEALAND

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## Introduction

The annual total production of Greenshell™ mussels in New Zealand has remained at around 100,000 tonnes for the past ten years. This industry continues to struggle with improving the efficiency of utilisation of early juvenile mussels or spat, that are used for seeding long-line mussel farms in coastal waters. The losses of mussels between seeding farms with spat and harvesting adult mussels is estimated to exceed 99% for a variety of reasons that have proven difficult to solve with on-farm husbandry. As a result, attention has moved toward using research to develop contained nursery culture of mussel spat to raise them to a larger and more resilient sizes, prior to seeding out onto coastal mussel farms for on-growing to harvest size. However, the nursery culture of mussel spat in containment does not have an international precedent that can be followed, either for mussels or other aquacultured shellfish species. This has led to a multi-pronged research programme aiming to overcome some of the many challenges associated with contained nursery culture of mussel spat, including sourcing spat, feeding, contained nursery culture systems, handling and husbandry. This presentation will outline the problems faced by the Greenshell™ mussels in New Zealand, the breadth of research activity that has resulted, and the progress of the research toward resolving the challenge of developing an effective nursery culture system to improve aquaculture production of this species.

# POTENTIAL OF THE AMPHIPOD *Elasmopus rapax* FOR USE IN AQUACULTURE SYSTEMS

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## Introduction

The use of biological agents to control parasitic diseases in aquaculture is becoming increasingly popular, as it is considered a safe, effective, and economically beneficial strategy for sustainable aquaculture practices (Shirakashi & Takagi, 2021). Monogeneans are among the most problematic parasites in fish farms. Among them, *Neobenedenia* spp. severely affects *Seriola dumerili*, largely due to the accumulation of eggs in farming structures. The elimination of these eggs using biological agents, such as the gammarid *Elasmopus rapax* may be a novel solution for controlling outbreaks. Previous results obtained at our facilities have shown high consumption of *Neobenedenia*'s eggs by *E. rapax*, indicating that it may be a useful biological agent for removing monogenean eggs from culture facilities (Marrero et al., 2024).

On the other hand, *E. rapax* shows great potential as an alternative live feed for the larvae of species of aquaculture interest, due to its size and nutritional composition, especially its high content of essential fatty acids. However, the culture of amphipods, and specifically of *E. rapax*, has been poorly studied, so it is necessary to improve its mass culture to benefit the aquaculture industry (Suhaimi et al., 2024). Therefore, it is important to obtain information on both the culture parameters that may influence growth, development, and survival of juveniles, as well as the reproductive biology of *E. rapax*. So, it is important to provide information on *E. rapax* culture parameters such as density, culture method and feeding strategy. Among these parameters, the use of different food combinations and photoperiod regimes may provide valuable information on *E. rapax* growth and survival, and are relevant for mass culture protocols.

## Material and methods

Specimens of *E. rapax*, abundant on the walls of outdoor culture tanks at the facilities of the Oceanographic Center of the Canary Islands (COC-IEO), CSIC (Canary Islands, Spain), were collected from the surface of the tanks and kept in plastic containers with biofouling and sessile organisms. As *E. rapax* is commonly found among algae in the shady areas of the tanks, we tested three food sources: (1) eggs of *N. melleni* (Eggs), (2) algae that occur naturally in COC tanks (Algae), and (3) a mix of both diets (*N. melleni* eggs + algae) (Mix), combined with two photoperiod regimens (12L:12D and 4L:20D). To this end, 8 females at a similar stage of maturation were selected and kept individually, being monitored daily. When juveniles were observed, 6 offspring of *E. rapax* newly released from the females' brood chambers, were selected, and placed in each Petri dish (9 cm in diameter, 1.5 cm deep) with 40 ml of seawater (by triplicate). The Petri dishes were kept at 25°C, and monitored for 34 days in order to determine survival and growth.

To determine the optimal culture density of *E. rapax*, a second experiment was conducted, consisting of four treatments with different initial densities in Petri dishes (50 cm²): 1, 5, 8, and 15 ind/dish (by triplicate). The plates contained nylon mesh discs as a substrate, and *N. melleni* eggs as a food source, and 40 ml of seawater. The survival was monitored daily for 5 days. In both experiments the water and food (eggs, algae and mix) were replaced daily. The *E. rapax* were counted, photographed and measured using ImageJ software (NIH). The eggs of the parasite *N. melleni* used for the experiments were collected daily (every 24 h) from the *S. dumerili* rearing tanks, washed thoroughly, and used immediately for the experiments, after estimating their number under a stereomicroscope.

## Results

Survival and growth of *E. rapax* was affected significantly by food source and photoperiod regimen (Fig. 1). In all treatments, mortality began at 20 days, except for amphipods fed algae under 12L:12D, which began at 15 days. Regardless of the photoperiod, the highest final survival occurred when amphipods fed eggs, and the worst in those fed algae.

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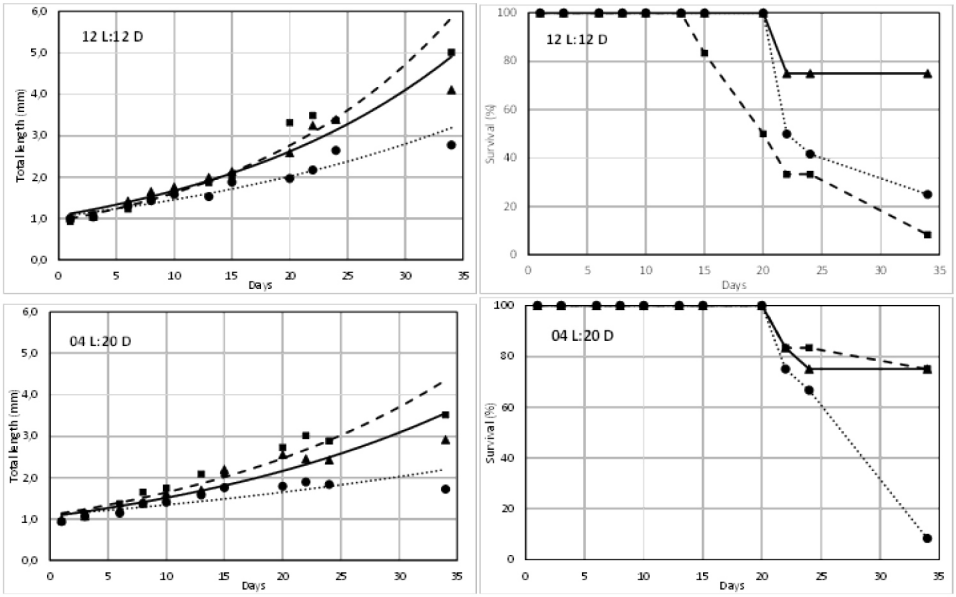


**Figure 1.** Total length (mm) and survival (%) of *E. rapax* under the experimental treatments of food (Eggs (–▲–), Algae (▪●▪), Mix (–■–). and photoperiod (12L:12D and 4L:20D).

The size of *E. rapax* kept under 12L:12D was greater than under 4L:20D. However, this difference was only significant ($p<0.05$) in those fed with algae. Regardless of the photoperiod, gammarids fed the algae-based diet were significantly smaller than those fed the egg-based or mixed diets, which showed similar growth.

On the other hand, the second experiment showed that the survival rate of treatments with initially 5, 8, and 15 ind/plate was similar after 5 days (73, 71, and 60%, respectively). Furthermore, regardless of the initial density, 25% of mortality occurred at 24 h.

**Discussion and conclusions**

Although *E. rapax* is commonly found among algae in low-light areas, where it protects itself and feeds, the present results show that it is possible to keep them with monogenean parasite eggs without special lighting conditions. However, adequate culture density would minimize mortality in mass cultures.

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# TURNING EFFLUENTS INTO HIGH-VALUE BIOMASS: A NEW FRONTIER IN CORVINA AQUACULTURE

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Integrated Multi-Trophic Aquaculture (IMTA) offers a sustainable strategy for modern aquaculture by combining species from different trophic levels to optimize nutrient recycling and reduce environmental impacts. This study evaluated the integration of the polychaete *Hediste diversicolor* in a Recirculating Aquaculture System (RAS) producing corvina (meagre - *Argyrosomus regius*), using as input a sludge effluent originally intended for discharge. Three polychaete stocking densities (250, 500, and 1000 ind/m$^2$) were tested over 35 days, with stable environmental parameters monitored throughout the trial.

*H. diversicolor* exhibited high survival (>93%) in all treatments, with no significant differences in growth performance, suggesting that higher densities increase biomass yield without compromising individuals. Proximate composition showed stable protein and lipid content, while carbohydrate levels increased with density, indicating the feasibility of dense cultivation for valuable biomass production. Fatty acid analysis highlighted the species' biosynthetic capacity: despite being fed nutritionally poor sludge (EPA: 0.04%, DHA: 0.23%, n-3/n-6: 0.5), enriched levels of essential fatty acids were recorded (EPA: 13.4–14.5%, DHA: 2.2–2.5%, n-3/n-6 ~1.8). This demonstrates the ability of *H. diversicolor* to convert organic waste into nutritionally valuable biomass.

Beyond its use as fishing bait, *H. diversicolor* shows potential for aquafeeds and broodstock nutrition (particularly for *Solea senegalensis*, where its nutritional profile and natural odorants may stimulate feeding and enhance reproductive performance). This innovative system provides a promising model for integrating high-value species into corvina RAS production, reinforcing IMTA as a tool for effluent remediation and circular resource valorization in sustainable aquaculture.

# RETHINKING AQUACULTURE FEEDS: SENSORY IMPACT OF ALTERNATIVE PROTEINS IN GILTHEAD SEA BREAM (*Sparus aurata*)

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## Introduction

The growing demand for sustainable aquaculture practices, driven by the rapid expansion of global aquaculture production, has highlighted the need to reduce reliance on fishmeal and fish oil as primary ingredients in aquafeeds. This has prompted the exploration of alternative protein sources that can support both fish growth and product quality. Traditionally, plant-based ingredients of terrestrial origin have been used as substitutes in grow-out feeds for marine species. Moreover, current formulations with high levels of plant-derived meals and oils have proven suitable for the present genetic variability of gilthead sea bream populations selected for different growth potentials. However, the limited availability of these raw materials and increasing competition from other industries underscore the urgency of developing and testing new, more sustainable feed formulations. These include insect proteins, processed animal proteins, microbial proteins, micro- and macroalgae extracts, and by-products or discards from aquaculture and other animal and plant production systems.

Sensory analysis plays a crucial role in evaluating food quality, particularly in fish products, where sensory attributes directly influence consumer acceptance and market value. Attributes such as appearance, odor, flavor, and texture are especially important, as they are strongly affected by various factors, including species and especially diet composition. Changes in feed formulation—such as replacing traditional marine-based ingredients with alternative protein sources—may alter the organoleptic characteristics of the final product. Therefore, combining descriptive sensory analysis by trained panels with consumer studies is essential to understand both the objective sensory differences among fish fed different diets and their subjective acceptance by end consumers.

Considering the above, the aim of this study was to explore the effect of feeding gilthead sea bream (*Sparus aurata*), sampled at a final weight of 800 grams, with alternative protein sources, compared to a fishmeal-based diet, on its sensory profile and consumer acceptance.

## Materials and methods

The gilthead sea bream were fed the control (CTRL: fish meal-based diet) and two experimental diets (PAP: processed animal protein-based diet, and ALT: alternative protein-based diet) at the IATS aquaculture facilities, from an initial weight of 10–15 grams until they reached market size (800 grams), without any changes in growth rates or feed conversion efficiency. A trained panel from the research group Food Quality and Safety Group (Universidad Miguel Hernández de Elche, UMH Orihuela, Alicante, Spain) conducted the descriptive sensory analysis in one session. A sample group of 80 consumers was recruited at UMH (Spain). The consumer study was conducted at the UMH facilities in Orihuela. A penalty analysis was conducted on the affective data to identify which sensory attributes had the greatest negative impact on overall liking scores.

## Results

The trained panel detected significant differences ($p < 0.05$) for the attribute 'color', with fish fed the ALT diet being evaluated as darker compared to the CTRL and PAP treatments. Differences were also observed for 'firmness', where the muscle of fish fed the CTRL diet was less firm, while no significant differences were found between fish fed the two alternative diets. In the consumer study, significant differences were found in the attributes 'fatty flavor', 'fat perception', and 'aftertaste'. Considering the first two attributes, consumers preferred, and therefore rated more highly, fish fed the PAP diet, whereas CTRL fish received the lowest scores. Regarding aftertaste, that of the CTRL group was the least liked by consumers compared to fish fed the alternative diets (PAP and ALT). Penalty analysis revealed that low intensity of attributes such as fat perception, fish flavor, and

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aftertaste negatively impacted the overall liking scores—by up to two points—in fish from the ALT and CTRL groups. In the case of PAP, the only penalizing attribute was juiciness, also due to low intensity. Overall, consumer preference favored fish fed the PAP diet, with 42.5% of participants selecting it as their most liked sample, compared to 27.5% and 30% for fish fed CTRL and ALT diets, respectively.

**Conclusions**

The results suggest that alternative protein sources can be used in gilthead sea bream diets without negatively affecting sensory quality. However, fish fed the PAP diet showed the most favorable sensory attributes and the highest consumer acceptance. These findings highlight the potential of processed animal proteins as a promising alternative to fishmeal in aquafeeds, combining sustainability with product quality.

# SUSPENDED CULTURE OF COCKLES (*Cerastoderma edule*) IN EARTHEN PONDS UNDER IMTA CONDITIONS

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## Introduction

Bivalve production plays an increasingly important role in European aquaculture, not only for its economic and social value but also for the ecosystem services these organisms provide, such as nutrient recycling and water quality improvement. The cockle (*Cerastoderma edule*) is particularly relevant in regions such as the Ria Formosa, Portugal, where it supports both traditional fisheries and aquaculture initiatives. However, conventional intertidal culture practices often present limitations, including high mortality and exposure to environmental variability. In recent years, suspended culture systems within earthen ponds have gained attention as a potential alternative, especially when integrated into Integrated Multi-Trophic Aquaculture (IMTA) frameworks. In such systems, bivalves benefit from continuous immersion and the constant supply of organic matter derived from fish feeding, which can enhance growth performance compared to intertidal plots. Moreover, IMTA approaches contribute to the circular use of resources, promoting both environmental sustainability and economic efficiency.

This study aimed to evaluate the feasibility of suspended cockle culture in earthen ponds under IMTA conditions by comparing two container types (rigid mesh bags and perforated circular trays), two culture depths, and different stocking densities. Beyond assessing growth and condition index, particular attention was given to how suspended cockle rearing within polyculture ponds can complement fish farming, improve resource efficiency, and provide insights into the balance between productivity and product quality.

## Material and methods

Two types of containers for suspended cockle culture were tested: rigid mesh bags and circular perforated trays with a 1-cm mesh size. Each tray contained four perforated containers (5-mm mesh) arranged as pie-shaped sections within the tray. The trays (40 cm in diameter and 10 cm in height) were stacked in groups of four. Only the second tray from the top and the bottom tray in each stack were stocked with cockles, allowing a comparison of culture at two depths (20 and 40 cm). Two stocking densities were tested for each container type: 2 kg $m^{-2}$ and 3 kg $m^{-2}$. The culture system consisted of six rigid mesh bags and six stacks of trays suspended from a long-line with floaters, arranged side by side with alternating densities. At both ends of the line, two supplementary rigid mesh bags and two tray stacks with the same density treatments were installed to replace sampled individuals.

The structures were cleaned once or twice per month, depending on the degree of fouling by macroalgae and other organisms. Cleaning of rigid mesh bags only required turning them over to maintain water circulation. In contrast, circular trays had to be replaced and washed with freshwater or dried in the sun before removing fouling organisms. Every month, five cockles from each replicate, density, and container type (rigid mesh bag and circular tray) were sampled for biometric measurements (length, width, thickness, and weight) and for condition index determination.

## Results and Discussion

Over the same experimental period (3.8 autumn/winter months), cockles reared in circular trays at 3 kg $m^{-2}$ reached a growth rate of up to 85 µm $day^{-1}$, attaining 27.83 ± 1.69 mm in length and 6.92 ± 1.30 g in weight at the end of the trial. Growth in rigid mesh bags was slower, with cockles reaching 25.22 ± 1.91 mm in length and 5.29 ± 1.05 g in weight at the same density (Figure 1). Biomass yield ranged between 1300 g $m^{-2}$ and 1900 g $m^{-2}$ for both circular trays and mesh bags, with no substantial effects of density or rearing depth.

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# UTILIZING ARTIFICIAL INTELIGENCE OBJECT RECOGNITION FOR ANALYZING AND TRACKING HEALTH OF ARTEMIA, ROTIFER, SHRIMP, AND FISH IN HATCHERIES

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**Introduction**

Fish and shrimp hatcheries world-wide use a variety of methods to monitor the growth of their animals and live feed. This is often a labor-intensive process which relies on subjective judgements of field operators to produce accurate results. The ability of artificial intelligence (AI) models to recognize specified objects in digital images is an established trait that could prove useful when applied to counting animals and live feed in hatcheries in addition to tracking the growth of their developmental stages. The aim of this study is to create an AI model, trained on images labeled by experts, along with digital support systems that can recognize and track Artemia developmental stages, rotifers, rotifer eggs, early shrimp developmental stages, fish eggs, and fish egg quality for use in hatcheries around the world.

**Materials and Methods**

Samples containing examples of the target animals and animal life stages were prepared in 3x4 cell culture well plates. Images of these wells were taken using a digital camera and lens system that uploaded the files into a computer vision annotation tool (CVAT). The labels used in this image library include Artemia embryos, Artemia umbrellas, Artemia nauplii instar I, Artemia nauplii instar II, live rotifer, dead rotifer, rotifer eggs, shrimp nauplii, zoea, mysis and PL stages, live and dead seabass and seabream eggs. The labels were applied manually by experts and the AI model was trained using this dataset. A separate labeled dataset was used to verify the training of the model. The model is derived from a You Only Look Once (YOLO) object recognition base. This was chosen due to its speed and accuracy in tandem with a proper microscope-optics setup. The model was trained recursively via comparison to the known result of the separate set until a maximum accuracy asymptote was reached. This trained model was then installed with proper optics on a custom device named the SnappArt 360 to be tested in the field by technicians. The technicians reported feedback, and the model was trained several more times based on this cycle of labeling, training, technician testing, and feedback until the technicians reported satisfaction with the model accuracy. A user-friendly data management system for the SnappArt 360 machine was also created to let technicians track trends in animal growth patterns from past measurements.

**Results and Discussion**

Raw counts per sampled well (250µL sample volume) of Artemia nauplii, umbrella, and embryos were used to compare accuracy of the SnappArt AI model to the technician results for Artemia stage objects. Figure 1 shows the distribution of the differences between the technician and SnappArt measured values of these counts respectively. The average differences are 0.02, -0.03, and 0.03 with standard deviations of 1.00, 0.53, and 0.41 with no statistically significant difference in results between technicians and the SnappArt AI model according to p-Values of 0.728, 0.355, and 0.238 for nauplii, umbrella, and embryo counts respectively.

Results on other objects reflect the accuracy found in Artemia measurement with 95% of seabass and seabream eggs and over 95% of rotifers and rotifer eggs being identified with the SnappArt AI model under the correct annotation of live or dead. Figure 2 shows the accuracy of rotifer animal and egg counting.

Furthermore, results for shrimp show an accuracy of 99% with no statistically significant difference between technician and SnappArt AI counts for all larval stages of shrimp.

These results indicate that AI modeling could be a promising option for future automation of counting and monitoring the health of animals in hatcheries for a wide variety of species.

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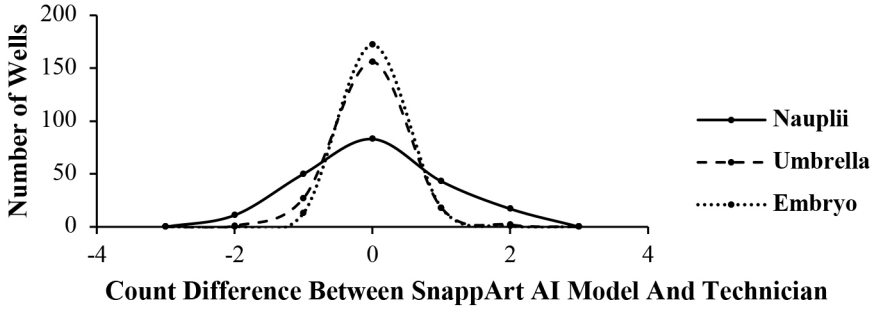


Fig. 1 Distributions of counting differences between SnappArt AI Model and technicians

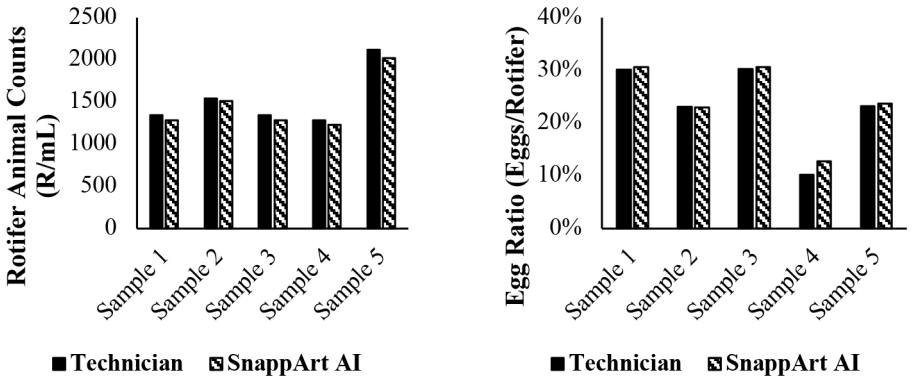


Fig. 2 Comparison of Rotifer animal and egg counting between technician and AI model.

# EFFECTS OF ENVIRONMENTAL ENRICHMENT ON BEHAVIOR AND GROWTH OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*) – RELEVANCE FOR FISH WELARE AND AQUACULTURE PRODUCTION

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## Introduction

Intensive aquaculture production challenges fish health and welfare, impacting both economic and ethical sustainability (Ashley, 2007). Increasing the complexity of the holding environment (environmental enrichment, EE) has been shown to have a range of positive effects on fish physiology and welfare, and is assumed to encourage the expression of adaptive behaviors (Gerber, 2015). How these advantages translate into an actual impact on central production parameters such as growth remains insufficiently documented, and EE is not yet broadly adopted in current aquaculture practices. This study investigates how early exposure to environmental complexity affects growth and welfare- related behavioral responses in juvenile rainbow trout.

## Material and methods

After 90 days of rearing in EE or BE, fish were transferred to individual isolation for two weeks, allowing for observations of differences in stress coping ability (Øverli, 2005).

## Results

Despite the fact that fish from EE vs B environments regained feed intake at a similar rate after transfer to a novel environment (figure 2 & 3A), mass gain was more than twice as high in fish transferred from EE compared to B rearing (figure 3B). Additionally, EE led to significantly lower frequencies of stereotypic vertical swimming following transfer to the novel environment (figure 4).

## Discussion

Findings from this study suggest that EE fish developed greater behavioral flexibility and resilience, based on the significantly lower frequencies of stereotypic vertical swimming following transfer to the novel environment (Mason, 1991; Prentice, 2025). The increased stereotypic behavior in BE fish after exposure to the novel environment suggests that growth differences are closely linked to stress coping behaviors in EE- and BE-reared fish.

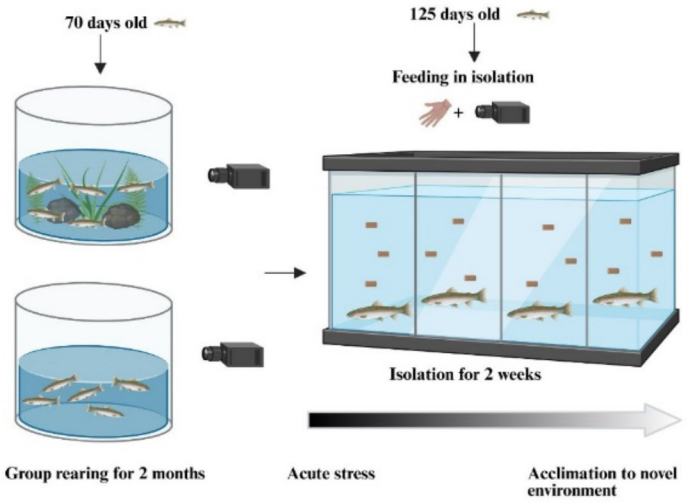


**Fig.1:** Overview of the experimental design. After 90 days of rearing in EE vs BE, juvenile rainbow trout (85.4 ± 3.54 g) were sedated, weighed and measured, transferred to isolation and handfed (limit 2% BW/ day) for two weeks.

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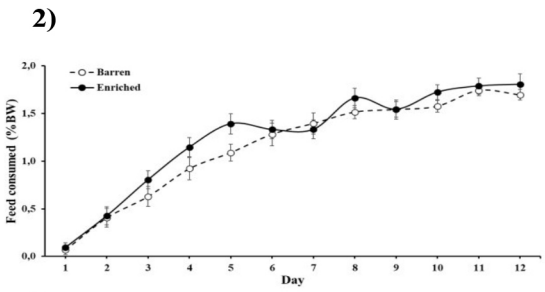


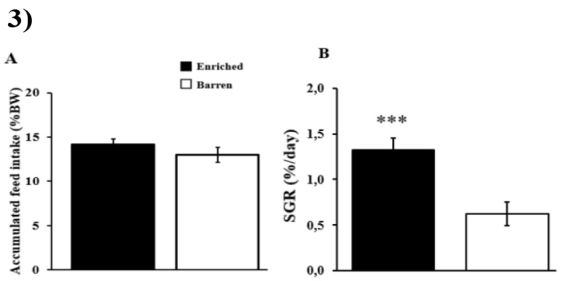


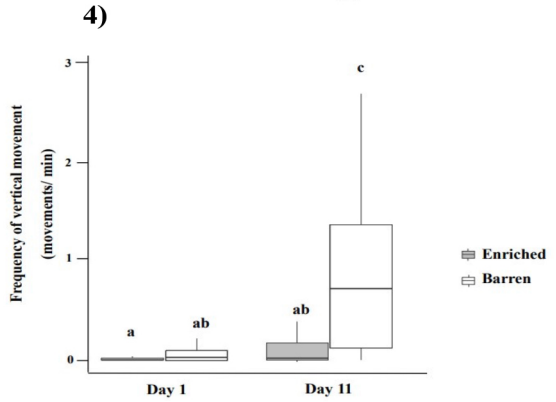


**Fig. 2:** Accumulated feed intake (%BW) over 12 days in juvenile rainbow trout after transfer to a novel environment showed no significant effect of EE.

**Fig 3:** Feed efficiency during acclimation to a novel environment in juvenile rainbow trout reared in EE and BE tanks. **A)** Feed intake (%BW) unaffected by rearing environment. **B)** SGR (%/day, mean ± S.E.M) significantly higher in EE fish.

**Fig 4:** Stereotypic vertical movement in juvenile rainbow trout reared in EE and BE tanks on day 1 and 11 after transfer to isolation; significant interaction effects indicated by lowercase letters.

## Conclusions

These results point to the value of broadening behavioral indicators used in aquaculture, as behavioral outcomes of welfare translate, to some degree, directly to growth performance following environmental change. This observation should serve as a strong motivation to maintain a clear focus on welfare status in aquaculture production.

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# FOOD PRODUCTION USING RECOVERED WATER AND NUTRIENTS THAT INTEGRATES WETLAND EFFLUENT TREATMENT, VEGETABLE AND FISH PRODUCTION – A PROGRAM OVERVIEW

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**Introduction**

Freshwater is becoming increasingly scarce and valuable, and agriculture and agro-processing sectors remain among the world's largest users of this resource. This program has integrated various nature-based technologies and processes, e.g., algal ponding, constructed wetlands, hydroponics, aquaponics, vermiculture and permaculture to recover water and nutrients from effluent produced by agro-processing and to do so using a circular economy to generate agriculture and aquaculture products. The proof-of-concept was established within the first few years of the program. Algal ponding and wetland technologies were integrated with hydroponics and aquaponics with a focus on optimising the anaerobic digestion phase of the treatment process; effluent was successfully cleaned, crops were produced, and this was all done using an integrated effluent treatment/aquatic food production system. The next decade was designed to obtain societal support to scale up the project, while also further developing and optimising the process.

**Recent research progress**

The presentation will delve briefly into the progress that has been made in the following areas of research, all of which have supported the development of this alternative effluent treatment and food production system in an aquatic environment:

- A sustainable, nature-based method of "harvesting" microalgae from treated effluent using filter-feeding fish;
- Comparison of different kinds of constructed wetlands and their effect on effluent treatment and vegetable production;
- The addition of nanobubble and ebb-and-flow wetlands to increase treatment capacity of the constructed wetland;
- Optimising wetland filter media type, size and hydraulic retention time;
- Developing measures to mitigate the buildup of salts to optimise crop production, as effluent is recycled;
- Preliminary look at the inclusion of fish production in the system; and finally
- Obtaining societal support to demonstrate the process on a commercial scale.

**Culmination of this work**

This alternative wetland-based system has been successfully integrated with hydroponics, vermiculture, permaculture and aquaponics, and has been tested on a commercial-pilot scale for the last three years. Approximately 350 $m^3$ of brewery effluent is treated daily and about 250 $m^3$ of cleaned water is returned to the brewery. Post anaerobically digested brewery effluent enters the system with a chemical oxygen demand (COD) of 400-600 mg/L and leaves at 60-80 mg/L. Total ammonia enters at 40-50 mg/L and is discharged at < 1 mg/L. This integrated effluent treatment process produces over 2-tons of fresh vegetables per month, supporting seven farm workers, and this fresh-produce is used to support local feeding schemes and is sold to supermarkets.

**Way forward**

The next steps will be to develop partnerships so that we can demonstrate this approach using different effluent streams, in different geographical regions, using alternative crops and optimising the process for a more universal application. Future work will also look at including a finfish loop in the system to add nitrogen and additional, valuable protein-rich products because the primary effluent source can be nitrogen limited. The addition of aquaculture effluent might reduce the need for the addition of inorganic fertilizers needed to optimise plant production, but this remains to be tested.

# EFFECT OF FEEDING SYSTEM USING TIMER OR ACOUSTIC FEEDERS, AND TRANSFER WEIGHT ON PERFORMANCE AND ECONOMIC PROFITABILITY OF WHITE SHRIMP (*Penaeus vannamei*)

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## Introduction

White shrimp is the most important species from an economic point of view of world aquaculture, which is produced in semi-intensive ponds, with two production phases, pre-growing and growing until commercial size. Recently, the use of automatic feeders, timed and acoustic, has increased in commercial farms (Valle et al., 2023; 2025), with a positive effect on performance, but the recent trend for increasing transfer weight of shrimp from pre-growing to growing ponds requires an evaluation of performance and profitability performance for optimizing shrimp production.

## Material and Methods

Data from 240 production cycles from four different shrimp farms of Guayas region (Ecuador) were studied. Size of ponds ranged between 1.7 and 22.7 has. Initial density varied between 7 and 18 shrimp per square meter. Two automatic feeding systems were analyzed, time feeding during 12 hours a day, from 12.00 to 24.00 hours, and demand feeding with acoustic feeders. Shrimp transfer weight to growing pond ranged from 0.3 g to 11.2 g, but they were grouped for analyzing in five intervales, ≤ 1g, ≤ 2g, ≤ 4 g, ≤ 8 g and ≤ 12 g. ANOVA of one way and two ways were used for statistical analysis data of final weigh, survival, production (kg/ha), food conversion ratio (FCR), days of cycle, growth (g/week), and economic profit (US$/ha, and US$/ha/day), using LSD test for comparing the means.

## Results and discussion

Shrimp farms and size of ponds do not have a significant effect on performance or profit. Results of shrimp fed with timer feeder and acoustic feeder are presented in Tables 1 and 2 respectively. Final weight and survival were not present statistical differences, with mean values of 31.6 g and 30.9 g, and 78.5 % and 30.9 %, respectively. Production (kg/ha) was positively related to density, but $R^2$ was very low, 20%.

The low transfer weight (≤ 1g and ≤ 2g) originated a longer production cycle, lower weekly growth, and lower economic profit in both feeding systems, and a worse FCR but only with time feeder. Production was higher with a transfer weight ≤ 8 g with time feeders, but similar for all transfer weight with acoustic feeders.

Global profit of shrimp production with acoustic feeders was statistically higher than with timer feeders, 1906 vs 927 US$/ha and 32.3 vs 14.7 US$/ha/day (data not shown), but the effect for several transfer weight was different. Feeding systems did not present statistical differences on economic profit when transfer weight was ≤ 1 g, ≤ 4 g and ≤ 8 g, but when transfer weight was ≤ 2g, acoustic feeding origin a higher profit than timer feeding (Table 3). Likewise, some differences appeared for transfer weight ≤ 12 g.

Results are not definitive, because data with high transfer weight are scarce for comparing, and more data for evaluating technical performance and economic profit should be needed, but as preliminary conclusion, a transfer weight between 4 and 8 g with timer feeders and between 2 and 4 g with acoustic feeders, could be adequate.

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Table 1: Performance and profit of shrimp fed with time feeders

| Transfer weight (g) | Production (Kg/ha) | Growth (g/w) | Days Cycle | FCR | Profit (US$/ha) | Profit (US$/ha/d) |
|---|---|---|---|---|---|---|
| ≤ 1 (105) | 2827 bc | 2.7 d | 80 b | 1.54 c | 701 b | 10.5 b |
| ≤ 2 (51) | 3003 ab | 2.8 c | 77 b | 1.56 c | 889 b | 12.9 b |
| ≤ 4 (6) | 2610 abc | 3.3 a | 60 a | 1.37 ab | 1492 ab | 26.5 ab |
| ≤ 8 (17) | 3147 a | 3.0 ab | 60 a | 1.53 bc | 2138 a | 38.5 a |
| ≤ 12 (5) | 2358 c | 2.8 bcd | 49 a | 1.26 a | 1287 ab | 26.8 ab |
| Signif Level | 0.0293 | 0.0000 | 0.0000 | 0.0007 | 0.1307 | 0.0057 |

Means with different letters are statistically different (LSD test)
Number of data is presented in brackets.

Table 2: Performance and profit of shrimp fed with acoustic feeders

| Transfer weight (g) | Production (Kg/ha) | Growth (g/week) | Days Cycle | FCR | Profit (US$/ha) | Profit (US$/ha/d) |
|---|---|---|---|---|---|---|
| ≤ 1 (26) | 3047 | 2.6 c | 84 c | 1.48 | 896 b | 12.9 b |
| ≤ 2 (10) | 3280 | 2.8 bc | 70 b | 1.43 | 2329 ab | 35.3 ab |
| ≤ 4 (10) | 3365 | 3.0 b | 59 a | 1.34 | 3432 a | 59.0 a |
| ≤ 8 (8) | 3022 | 3.5 a | 51 a | 1.41 | 2556 ab | 52.7 a |
| ≤ 12 (2) | 2919 | 3.1 abc | 50 a | 1.34 | 2685 ab | 53.9 ab |
| Signif Level | 0.5513 | 0.0000 | 0.0000 | 0.4447 | 0.1012 | 0.0136 |

Means with different letters are statistically different (LSD test)
Number of data is presented in brackets.

Table 3: Comparison of economic profit of shrimp fed with time and acoustic feeders for each transfer weight

| Transfer weigth | ≤ 1 | ≤ 2 | ≤ 4 | ≤ 8 | ≤ 12 |
|---|---|---|---|---|---|
| **US$/ha** | | | | | |
| Time Feeder | 701 (105) | 889 b (10) | 1492 (6) | 2556 (17) | 1287 b (5) |
| Acoustic Feeder | 896 (26) | 2329 a (51) | 3431 (10) | 2138 (8) | 2685 a (2) |
| Significance Level | 0.7243 | 0.0268 | 0.1563 | 0.5522 | 0.0463 |
| **US$/ha/day** | | | | | |
| Time Feeder | 10.5 (105) | 12.9 b (10) | 26.5 (6) | 38.5 (17) | 26.8 (5) |
| Acoustic Feeder | 12.9 (26) | 35.3 a (51) | 59.0 (10) | 52.7 (8) | 53.9 (2) |
| Significance Level | 0.7356 | 0.0120 | 0.1791 | 0.3534 | 0.0892 |

Means with different letters are statistically different (LSD test).
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# IMPROVING PERFORMANCE OF WHITE SHRIMP *Penaeus vannamei* FED WITH BIOCOMPONENTS AND NANOENCAPSULATED ENRICHED DIETS

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## Introduction

White shrimp is a very important species in world aquaculture, because it means the most economic value. Nutrition is the key to production and in recent years the use of functional additives has been considered in diets, particularly *Bdellovibrio bacteriovorus* and other additives as bacterial biocontrol (Cao et al., 2019; Williams and Piñeiro, 2006; Harpeni et al., 2024; Wena et al., 2014; Hany et al., 2022; Hossain et al, 2024) and weight promoter (Li et al., 2014). The objective of this study was to determine the effect of diets enriched with some biocomponents and nano-encapsulated additives on white shrimp performance.

## Material and Methods

Four experimental diets supplemented with *B. bacteriovorus* (10 g/kg), Bacteriophage (10 g/kg), Bovine plasma (5 g/kg) and a Mixture of amino acids (0.07 g/kg methionine, 0.04 g/kg arginine, 0.05 g/kg tryptophan, 0.22 lysine, 2 g/kg glutamine, 0.5 g/kg taurine, 0.054 g/kg syli-grenade and 0.04 g/kg nano-grenade), and one commercial control diet were studied. Experimental diets were prepared from a commercial diet (22 % protein) by ground, water addition, drying and granulation (1 mm diameter). All diets were given twice with an initial feeding rate of 10 %, which was adapted based on daily consumption determining daily non-consuming feed.

Sixteen semi-conical 800 L fiber tanks (3 replicates for experimental diets and 4 for control diet) were used. Water operational volume was 400 L/tk and water exchange was 400% a day, using saline water with 33 ppt. The water temperature was 29-30 ºC, dissolved oxygen 6.1 ppm, ammonia 0.14 ppm and nitrite 0.03 ppm.

Fifteen shrimp weighting 1,01±0,12 g were installed in each tank, and they were fed during eight weeks.

Weight gain and survival results were analyzed by one way ANOVA, using Student-Newman-Keuls test for mean differences.

***Table 1. Performance of white shrimp fed with experimental diets.***

| Diet | Weight gain (g) | Survival (%) | Biomass gain (%) | Feed consumption (g) | FCR |
|---|---|---|---|---|---|
| Control | 4.3 bc | 96.7 | 431 ab | 242 a | 3.8 a |
| *B. bacteriovorus* | 6.0 a | 91.1 | 487 a | 306 d | 3.6 a |
| Bacteriophague | 5.4 ab | 86.7 | 358 b | 289 c | 3.9 a |
| Bovine plasma | 3.0 c | 82.2 | 207 c | 257 b | 6.2 c |
| AA mixture | 4.0 bc | 80.0 | 259 c | 265 b | 5.0 b |
| Signif. Level | 0.0021 | 0.3349 | 0.0001 | 0.0000 | 0.0002 |

Means with different letters are statistically different (S-N-K test) ($P$<0.05).

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## Results and discussion

Results of weight gain, survival, feed consumption, biomass gain, and food conversion ratio (FCR) are presented in Table 1.

Highest weight gain was obtained with *B. bacteriovorus* diet (6.0 g) and Bacteriophague diet (5.4 g), and lowest with Bovine plasma diet (3.0 g). Growth was low in all diets, less than 0.75 g/week, probably due to low dietary protein and space limitations in the tanks.

No differences in survival were obtained at the end of trial, but Control diet presented a high value, whereas Plasma and AA Mix had low value.

In general, FCR were very high, because the objective was sure the maximum ingestion of experimental diets instead of minimizing feed conversion ratio. Feed consumption was highest for *B. bacteriovorus* diet, and lowest for Control diet. No differences in FCR were observed for Control, *B. bacteriovorus* and Bacteriophague diets, but Plasma and Mix diet presented the highest value.

Results suggest that Bovine plasma and AA mixture diets had a negative effect in growth and survival, but *B. bacteriovorus* diet improved growth and biomass gain, which agrees with results obtained by Li et al. (2014) in *P. monodon* shrimp, although these authors used the additive in water instead of into the feed.

In conclusion, shrimp diet supplementation with *B. bacteriovorus* could be interesting for improving performance in clear water trials, but new studies are necessary in commercial farming conditions.

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# EXAMPLE OF AN UNCOUPLED AQUAPONIC FACILITY FOR EFFICIENT NUTRIENT AND WASTE MANAGEMENT

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## Introduction

Despite intensive research and operational innovation for over the past 30 years, "aquaponic" systems that combines fish culture and hydroponic plant production are still considered "experimental" by most authors. The effluent from a recirculating aquaculture system (RAS) includes ammonia, nitrate, nitrite, phosphorus, potassium, and other secondary micronutrients that are necessary to sustain hydroponic plants production (Rakocy et al. 2006; Kloas et al., 2015). There are basically two types of hydroponic systems. A "coupled" aquaponics system, uses the hydroponic biomass (specifically the submerged roots) as a large biofilter that extracts the nutrients from the wastewater, purifying it and returning it to the fish tanks for sustaining this secondary biomass. To be effective, coupled aquaponics systems must be sized exactly to balance the nutrient production from the fish culture with the potential for nutrient uptake by the plants. This balance can be precarious to achieve and maintain. For example, the nitrification process necessary for water quality maintenance is efficient at a pH of 7.0 or higher but practically ceases at less than 6.0. The optimum for nutrient solubility is 6.5. If pH is too high, nutrients precipitate out of solution and plants display nutrient deficiencies. There is minimal acceptable overlap of even this simple water quality parameter. In an "uncoupled" aquaponics system, the fish production and hydroponics systems are separate, and are designed independently. The recirculating aquaculture operation treats the water using biofiltration and solids removal technologies, resulting in elevated nitrate levels and the accumulation of solid wastes in the waste stream. These effluents can be processed in different ways to the benefit of a hydroponics operation. However, the wastewater leaving the fish culture operation is not returned to the fish tanks. There are various designs of each type of aquaponics system, some successful but often not, due to inadequate understanding and respect for either of the two basic activities: fish farming or hydroponic farming. This article describes the design and efficiency of a decoupled aquaponic system consisting of a closed recirculating aquaculture system, waste stabilization tanks, a constructed wetland, and a hydroponic greenhouse.

## Materials and Methods

The recirculating fish production system design includes concrete tanks, with a total volume of 120 $m^3$ of water. Hybrid striped bass were raised at the maximum stocking density of 45 $kg/m^3$. Each e tank was integrated with a water recirculation unit (Jug-Dujaković et al., 2010). Solids were removed from the aquaculture system by a micro-screen filter. The screen was backwashed at periodic intervals to discharge solids to a swirl separator, discharging between 5% and 8% of the fish culture system volume daily. The swirl-separator was used to separate settleable solids. Cleared supernatant was transferred directly to the hydroponic greenhouse. The concentrated solids were transferred to aerobic stabilization tanks where heterotrophic bacteria aerobically degraded and mineralized the solids for 3-4 weeks. These were then applied to the surface of a constructed wetland. Constructed wetlands are fabricated inside a concrete basin, with layers of coarse and fine gravel, topped with a reed bed of Phragmites. The periodic discharge from aerobic stabilization tanks was filtered through the constructed wetlands and the clear nutrient-rich water was transferred to the hydroponic greenhouse, the elevated nutrient levels available to a variety of hydroponically cultured vegetables. Maximal planting density was 30 plants per $m^2$. During the hydroponic growth cycle, the plants purified the water by utilizing the dissolved nutrients for their growth. The greenhouse area was scaled to efficiently extract the nutrients from water discharged from the recirculating aquaculture system. The clear treated water could then be sterilized with ozone and reused within the fish culture facility or discharged. After years of accumulation, the solids on the surface of the reed beds are removed and used as fertilizer.

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**Results and Discussion**

The aquaculture process continuously generates both solid and liquid organic waste. Liquid waste includes nitrates a by-product of the biofiltration process) and other dissolved nutrients within the culture water. Approximately 25% of the feed given to fish is excreted as solid waste. If not stabilized the organic waste has a characteristic unpleasant smell, and since it is not mineralized and dissolved in water, it is mostly unusable for growing plants. Also, if solids are not properly removed, they will adhere to plant roots, decrease oxygen levels as they decompose and have an adverse effect on nitrifying bacteria (Jug-Dujaković et al., 2010). A substantial quantity and variety of organic vegetables can be cultured using the dissolved waste from a RAS, creating a secondary profit center. Those plant varieties studied and confirmed as appropriate for this process include various types of lettuce, water crest, tomatoes, cucumbers and herbs. The measured average levels of macronutrients in the waste stream from the mechanical drum filters and from the hydroponic greenhouse effluent demonstrates reduction of N-NO3 from 165 to 8.4, N-NO2 from 0.84 to 0.56, and P-PO4 from 8.2 to 0.7 ppm. Waste mineralization and water quality of the system was maintained at a feeding rate of 90 g of striped bass feed/$m^2$/day for mixed vegetable production, which is in agreement with data from Rakocy et al. (2006). Gloger et al. (1995) reported that the raft hydroponic tanks removed an average of 0.56 g of total ammonia-nitrogen, 0.62 g of nitrite-nitrogen, 30.29 g of chemical oxygen demand, 0.83 g of total nitrogen and 0.17 g of total phosphorous per m2 of plant growing area per day using romaine lettuce.

In conclusion, a decoupled aquaponic system designed with appropriate pretreatment of wastewater before sending it to the hydroponic greenhouse was successful in producing fish and plants creating two profit centers, while effectively reducing aquaculture wastewater.

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# ELUCIDATING THE IMMUNOREGULATORY ROLE OF NLRC5 IN VIRAL INFECTION

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## Introduction

The growth of the aquaculture industry is being hindered by increasing mortality caused by infectious diseases and environmental stressors. In particular, viral infections cause significant damage in aquaculture fish due to the lack of effective treatments. Therefore, understanding and studying the antiviral immune system of fish is essential to deal with viral diseases.

NLRC5 (NOD-like receptor family CARD domain containing 5) belongs to the NOD-like receptor family and is known to be involved in immune responses by regulating histocompatibility complex (MHC) I gene expression and type 1 interferon in mammals, but its role in teleost remain unclear. In this study, we established an Nlrc5 knockout (KO) zebrafish model to investigate the role of NLRC5 in the antiviral immune response of teleost, and demonstrated its importance through viral hemorrhagic septicemia virus (VHSV) infection experiments.

## Materials and methods

Nlrc5 KO zebrafish were generated using the CRISPR/Cas9 system. VHSV infection was induced in both wild-type (WT) and Nlrc5 KO zebrafish via intraperitoneal (IP) injection ($1\times10^6$ and $5\times10^6$ $TCID_{50}$/fish), and mortality rates, viral replication, and downstream gene (cytokines, class-I MHC pathway components, neutrophil markers) expression were subsequently compared.

## Results and discussion

CRISPR/Cas9-mediated Nlrc5 knockout zebrafish were identified to have a 10 bp deletion sequence at the sgRNA target site, resulting in a frameshift mutation that produced a truncated 206-amino acid peptide. The mutated protein contained only the CARD domain, while other functional domains, including the NACHT domain, were completely absent.

In the VHSV infection experiment using WT and Nlrc5 KO zebrafish, the Nlrc5-deficient fish exhibited higher mortality, increased virus replication, and severe pathological symptoms (including hemorrhage, and eye extrusion). Gene expression analysis following VHSV infection revealed that ifnϕ1 was significantly upregulated, while expression of cytokines involved in neutrophil migration and MHC-I genes was significantly downregulated in Nlrc5 KO zebrafish compared to WT zebrafish. In addition, neutrophil migration analysis confirmed reduced neutrophil recruitment to the caudal fin injury site in Nlrc5 knockout zebrafish. These results demonstrate that Nlrc5 is essential for the antiviral immune response to VHSV infection in zebrafish, primarily through regulation of the MHC class I pathway.

Collectively, this study provides evidence for the role of Nlrc5 in antiviral immunity in teleost, enhancing our understanding of innate immune regulation and highlighting its significance in antiviral immune system.

# EMBRYOTOXIC EFFECTS OF CONTAMINATED WATERS FROM WAR ZONES ON *Danio rerio* in EARLY DEVELOPMENT

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The ongoing military conflict in Ukraine has led to substantial ecological degradation, including the contamination of freshwater systems. To evaluate the potential developmental toxicity associated with aquatic pollution in conflict zones, six surface water samples were collected from four affected regions: Kyiv, Chernihiv, Kharkiv, and Odessa. The present study assessed the impact of these water samples on early developmental stages of *Danio rerio*, a widely accepted vertebrate model in ecotoxicological research. Embryonic spontaneous tail coiling at 24 hours post-fertilization (hpf) showed no significant differences among treatment groups, suggesting the absence of acute neurodevelopmental toxicity. Heart rate (HR) analysis at 48 hpf indicated a physiological response to waterborne exposure; however, HR values did not significantly differ from those of the control group ($p > 0.05$). No statistically significant changes in HR were observed at 96 hpf. In contrast, a significant reduction in hatching rates was observed at 72 hpf among treatment groups ($p < 0.05$), although this effect was no longer apparent at 96 hpf ($p > 0.05$). These findings suggest that exposure to water from war-impacted regions may induce transient developmental delays, particularly in cardiophysiological function and hatching. The results underscore the need for further investigation into the long-term ecotoxicological consequences of armed conflict on aquatic organisms and freshwater biodiversity.

**Acknowledgement**

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# PRELIMINARY COMPETITIVE ELISA-BASED SCREENING OF TETRODOTOXIN OCCURRENCE IN BIVALVES FROM KOREAN WATERS

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## Introduction

Tetrodotoxin (TTX) is a potent neurotoxin that blocks voltage-gated sodium channels, disrupting nerve conduction and causing muscle paralysis, respiratory failure, and potentially fatal outcomes in humans at doses as low as 1–2 mg. Initially isolated from pufferfish (genus *Takifugu*), TTX has since been detected in various marine organisms, including gastropods (mud snails, trumpet shells), cephalopods (blue-ringed octopus), and crustaceans (xanthid crabs). Recent detections of TTX in European bivalves—species historically considered free of this toxin—have raised significant food safety concerns and underscored the importance of continuous monitoring. The occurrence of TTX in bivalves is attributed to bioaccumulation through filter feeding on TTX-producing microorganisms, with environmental changes potentially facilitating the spread or migration of these microorganisms into new habitats. Despite these developments, data on TTX occurrence in bivalves from Korean waters remain scarce. To address this gap, this study conducted a preliminary screening to investigate the presence and spatial distribution of TTX in both wild and farmed bivalves from Korean waters.

## Materials and Methods

Bivalve samples were collected from 10 coastal regions of Korea, encompassing the west, south, and east coasts, and comprising 11 species, including ark cockles (*Anadara kagoshimensis*), oysters (*Crassostrea gigas* and *Saccostrea kegaki*), mussels (*Mytilus galloprovincialis*, *M. coruscus*, and *Arcuatula senhousia*), clams (*Ruditapes philippinarum*), and scallops (*Mizuhopecten yessoensis*, *Argopecten irradians*, *Chlamys farreri*, and *Chlamys* sp.). At each site, one to three individuals per species were measured for shell length and whole-body wet weight, after which 1 g of homogenized whole-body tissue was analyzed for TTX using a EuroProxima competitive ELISA kit (R-Biopharm Nederland B.V.) with a mouse monoclonal anti-TTX antibody. The assay, validated for TTX screening (limit of detection: 9.4 μg/kg; detection capability: 20 μg/kg), followed the manufacturer's protocol, which involved tissue homogenization, buffer extraction, centrifugation, and competitive binding with TTX-precoated microplates. Colorimetric detection was performed at 450 nm after substrate development, and TTX concentrations were determined from standard curves generated using RIDASOFT Win software (R-Biopharm, Darmstadt, Germany).

## Results

TTX was detected at two sites on the west coast in wild farrer's scallop (*C. farreri*) from Incheon and Sinan. The TTX level in farrer's scallop from Incheon was below the safety limit recommended by the European Food Safety Authority (44 μg/kg), whereas scallops from Sinan contained 51.6–125.5 μg/kg, exceeding the limit. On the south coast, TTX was detected in wild Mediterranean mussels (*M. galloprovincialis*) and wild scallops (*Chlamys* sp.) from Wando, with only one scallop specimen exceeding the limit. In Jeju, TTX was detected in *S. kegaki* and *R. philippinarum*, with *S. kegaki* below the limit but two *R. philippinarum* specimens exceeding it (48.2 and 63.7 μg/kg). On the east coast, TTX was detected only at Gangneung, where wild *M. galloprovincialis* contained up to 166.5 μg/kg.

## Discussion

Consistent with numerous previous reports, TTX is not restricted to traditionally recognized TTX-bearing taxa, but has also been identified in bivalve species. To our knowledge, the present study provides the first evidence of TTX occurrence in bivalves from Korean waters. The potential influence of marine environmental shifts, including elevated seawater temperatures, on toxin bioaccumulation in these organisms remains unclear. Consequently, continued seasonal and long-term surveillance are warranted to safeguard seafood safety and to enhance understanding of the spatial distribution of the toxin within Korean coastal ecosystems.

# IMPACT OF FEEDS CONTAINING ALGAE OIL ON INTESTINAL MICROBIOTA AND FILLET QUALITY OF GILTHEAD SEABREAM (*Sparus aurata*)

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**Introduction**

This study is part of a larger project to improve the nutritional properties of freshwater and marine fish. The aim is to ensure the effective transfer of high-quality nutrient molecules from the processing of the raw material to the final product through the use of innovative, fortified feeds. The final aim is to deliver fish products with optimal nutritional value that fully meet consumer expectations.

Within this framework, the present study experimentally investigated the effects of enriching feed with algal oil on the physiology and health of a marine fish: Gilthead sea bream (*Sparus aurata*). Key evaluation parameters included growth performance, fatty acid profiles in the fillets, gut microbiota composition and intestinal short-chain fatty acid (SCFA) profile.

**Materials and methods**

The experiment was carried out with 360 gilthead sea bream, distributed over nine tanks (three tanks per feeding group), over a period of 15 weeks. The fish were fed a commercial control diet and two experimental diets containing algal oil in two different quantities. At the end of the feeding trial, samples were collected to analyze the composition and functionality of the gut microbiota using high-throughput sequencing techniques previously described in publications by our group (Terova et al., 2021; Hasan et al., 2024; Rimoldi et al., 2025). In addition, gas chromatography was used in conjunction with mass spectrometry to quantify short-chain fatty acids (SCFAs) in the intestinal lumen and to evaluate the fatty acid profile of the fillets.

**Results**

The inclusion of algal oil in the experimental diet led to an enrichment of omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the fillets of sea bream. Consistent with this increase, the ratio of omega-3 to omega-6 PUFAs was higher in the sea bream fillets fed the experimental diets. Overall, the fatty acid profile in the fillets matched that of the diets, which we have previously observed in a similar study (Terova et al., 2021). While the lipid composition differed, the lipid content of the fillets remained similar in the different feeding groups.

The composition of the gut microbiota remained largely unchanged in the feeding groups, suggesting that the addition of algal oil had no dysbiosis effects on the autochthonous microbiota. A higher proportion of algal oil in the diet led to a decrease in a few families comprising potentially pathogenic species. Interestingly, the concentration of butyric acid in the intestinal lumen of the fish belonging to the experimental feeding groups was lower than in the fish of the control group. This could indicate that either the addition of algal oil influences the production of butyric acid or improves the absorption of butyric acid, resulting in a lower accumulation in the lumen.

**Conclusions**

This study demonstrates the importance of the feed used in fish farming beyond the growth performance and health of the fish. In particular, it emphasizes the role of feed formulations in shaping the nutritional quality of farmed fish for human consumption. We have found that the addition of algal oil to gilthead sea bream feed increases the content of omega-3 PUFAs, important nutrients for human nutrition, in the filet without any negative effect on the health of the fish, particularly on the status of the microbiota. Most importantly, these results provide a basis for the next phases of the I-FISH project, where different additives will be included in fish feed to improve the nutritional value of the final product, i.e., the fish filet. Understanding how different nutritional strategies affect gut microbiota, metabolic outcomes and filet composition is essential for the development of more sustainable and nutritionally valuable aquaculture products.

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# FISHMEAL REPLACEMENT BY *Ceratitis capitata* MEAL ON THE DIET OF GILTHEAD SEABREAM (*Sparus aurata*)

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**Introduction**

Sustainable development of aquaculture requires industrial aquafeeds that make a rational use of fishmeal and an increasing use of ingredients that are produced with a low environmental impact. Insect meals can serve as high quality proteins and sustainable ingredients towards sustainable aquafeeds (Gasco et al. 2022; Karapanagiotidis et al. 2023). The Mediterranean fruit fly (MFF, *Ceratitis capitata*) is a highly damaging crop insect pest for the management of which sterile insect technique (SIT) mass rearing programs are widely implemented worldwide (Colacci et al. 2025). From a nutritional point of view, it is a species with high protein and essential amino acid contents but so far has not been evaluated in fish diets. Thus, the aim of this study was to evaluate the dietary fishmeal replacement by graded levels of a *C. capitata* meal on the growth performance and fatty acid profiles of an important Mediterranean farmed fish species, the gilthead seabream (*Sparus aurata*).

**Materials and Methods**

A total number of 480 *S. aurata* juveniles of 6.5 g initial mean weight were obtained from a commercial fish hatchery, transferred to the aquaculture facilities of University of Thessaly and stocked at 16 tanks (125 L) in a closed seawater recirculation system. MFF with 56.6% of crude protein and 7.5% of crude lipid was supplied by Flying Spark (Israel). Fish in quadruplicate groups (30 fish/tank, 4 tanks/dietary group) were fed to satiety, twice a day, four isonitrogenous (49%), isoenergetic (21 MJ/Kg) and isolipidic (15%) diets. The control diet (CTRL) contained 25% of fishmeal, and in the rest three diets graded levels of MFF were included, viz. 7% (MMF7 diet), 14% (MMF14 diet) and 21% (MMF21 diet) corresponding to fishmeal protein replacement of the control diet at 20%, 40% and 60%, respectively. Water quality parameters were maintained at 21.0 ± 1.0 °C, pH at 8.0 ± 0.4, salinity at 33.0 ± 0.5 g/L, dissolved oxygen >6.5 mg/L, total ammonia nitrogen <0.2 mg/L and photoperiod was at 12:12h (light:darkness). After 12 weeks of feeding, fish were weighted to measure growth and feed utilization parameters. Four fish per tank (12 fish/group) were randomly selected for muscle fatty acid analysis and another 12 fish /group were selected for whole body proximate composition. Analyses were those described by Asimaki et al. (2025). Data were analysed using one-way ANOVA and differences between means were determined by Tukey's multiple-range test (P=0.05).

**Results and Discussion**

Results showed that survival, growth performance and feed utilization parameters of *S. aurata* fed the experimental diets were similar (P>0.05) among the fish groups (Table 1). This denotes that the dietary inclusion of *C. capitata* meal up to 210 g/Kg, replacing 60% of fishmeal protein, did not impair growth performance of *S. aurata* and feed utilization. The whole-body proximate composition of fish was unaffected by the diets (results are not shown) denoting that *C. capitata* meal was utilized/catabolized at a similar extent to fishmeal resulting in similar body nutrient deposition. The increased dietary inclusion of MFF meal significantly increased the muscle tissue levels of 18:2n-6 and total n-6 PUFA but did not exert an influence on the levels of DHA, EPA, n-3 PUFA and n-3/n-6 PUFA ratios. These encouraging findings recommend a deeper look on the use of *C. capitata* meal in fish diets as up to date no relative literature exists.

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**Table 1.** Growth performance, feed utilization and selected fatty acids (% of total) of *S. aurata* fed the experimental diets.

| Parameters / dietary groups | CTRL | MFF-7 | MFF-14 | MFF-21 |
|---|---|---|---|---|
| Growth & feed utilization | | | | |
| Survival (%) | 97.5 ± 1.7 | 99.2 ± 1.7 | 95.0 ± 3.3 | 96.7 ± 2.7 |
| FR (% BW/day) | 1.89 ± 0.05 | 1.95 ± 0.02 | 1.97 ± 0.04 | 1.96 ± 0.07 |
| FBW (g/fish) | 34.6 ± 1.6 | 34.4 ± 1.8 | 35.0 ± 1.8 | 34.2 ± 1.8 |
| TL (cm) | 12.5 ± 0.3 | 12.4 ± 0.2 | 12.5 ± 0.2 | 12.5 ± 0.3 |
| SGR (%/day) | 1.99 ± 0.06 | 1.98 ± 0.06 | 2.00 ± 0.06 | 1.98 ± 0.07 |
| FCR | 1.16 ± 0.03 | 1.20 ± 0.03 | 1.21 ± 0.05 | 1.21 ± 0.07 |
| PER | 1.75 ± 0.05 | 1.70 ± 0.04 | 1.68 ± 0.07 | 1.67 ± 0.09 |
| LER | 5.56 ± 0.15 | 5.38 ± 0.12 | 5.32 ± 0.21 | 5.44 ± 0.31 |
| HSI (%) | 1.26 ± 0.23 | 1.27 ± 0.12 | 1.29 ± 0.12 | 1.28 ± 0.26 |
| Selected fatty acids (%) | | | | |
| 18:2n-6 | 10.6 [a] | 10.7 [ab] | 10.9 [bc] | 11.1 [c] |
| 18:3n-3 | 2.5 | 2.5 | 2.4 | 2.4 |
| 20:5n-3 (EPA) | 4.4 | 4.2 | 4.2 | 4.2 |
| 22:6n-3 (DHA) | 11.2 | 10.7 | 11.3 | 11.6 |
| SFA | 27.0 | 27.1 | 26.2 | 25.9 |
| MUFA | 40.5 | 41.0 | 41.7 | 41.4 |
| Total n-6 PUFA | 12.5 [a] | 12.6 [ab] | 12.8 [ab] | 13.0 [b] |
| Total n-3 PUFA | 20.4 | 19.7 | 20.3 | 20.7 |
| n-3 / n-6 | 1.59 | 1.53 | 1.50 | 1.52 |

Note: Values represent means ± standard deviation of n=4. Values within each row not sharing a common superscript letter are significantly different ($P < 0.05$). FR, feeding rate; FBW, final body weight; TL, final total length; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; LER, Lipid efficiency ratio; HSI, hepatosomatic index.

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# PARTICIPATORY ASSESSMENT OF RESILIENT AQUACULTURE SYSTEMS IN NEPAL: HEALTH, WELFARE & SOCIOECOLOGICAL POTENTIAL

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**Background & Objectives:**
Smallholder aquaculture in Nepal supports thousands of livelihoods, but systemic barriers—such as inadequate inputs, improved genetic species, disease, and unreliable profitability margins—curtail its potential. This study examines aquaculture resilience, productivity, and welfare across 16 districts along the terai regions of Nepal, blending quantitative analysis from the National Development Research Institute baseline evaluation with farmerand market actor-led insights from 190 LWR-facilitated Participatory Rural Appraisal (PRA) sessions involving 4,023 farmers between January and June 2025.

**Methods:**
A mixed-method, non-experimental design was used to assess aquaculture systems across 16 districts. Data were collected from 1,003 fish farmers and 160 value chain actors using structured surveys, KIIs, and PRA tools (resource mapping, seasonal calendars, pair ranking). Carp yield (t/ha) was calculated using a farm-specific pond profile approach. A multistage stratified sampling strategy and Proportional to Population Size (PPS) estimates ensured representativeness. Data collection used Kobo Toolbox; analysis involved Stata and SPSS. Quantitative and qualitative findings were triangulated to evaluate ecological resilience, equity, and economic outcomes.

**Results:**
Productivity ranged from ~5.2 t/ha in nonfish zones to ~7.4 t/ha in fish superzones, aligning with national pond yield averages (~5.0–5.6 t/ha in 2022/23). Benefit–Cost ratios ranged from 1.3 to 1.7, with the highest returns on farms exceeding 0.5 ha in size, using integrated fishcroppoultry systems, and with access to credit. PRA groups consistently prioritized challenges such as seed quality, disease, feeding practices, credit access, technical knowledge gaps, and local market inefficiencies. Both PRA and survey data underscore that profitability correlates strongly with farm scale, credit access, integrated system usage, and feed-use efficiency.

**Conclusion & Significance:**
Feed comprises 40–50% of production costs, but nutrient recycling through integrated systems significantly reduces input dependence. Smallholders consume over 30% of their harvest, limiting the surplus for market sales and reducing income. Farmers with >10 years' experience and ponds larger than 0.5 ha achieved significantly higher returns. This study demonstrates that combining precision productivity measurement with participatory farmer diagnostics enables regenerative aquaculture pathways featuring integrated farming models, equitable input financing, and tailored extension services—offering replicable models for sustainable aquaculture and food-system resilience in Nepal and similar Global South contexts.

# ATLANTIC SALMON SCALE EXPLANTS IN BACTERIA-HOST INTERACTION STUDIES

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**Introduction**

Skin ulceration is a persistent issue in Norwegian salmon farming, adversely affecting fish welfare, increasing mortality rates, and diminishing product quality. The primary causes of skin ulcer formation are mechanical handling and bacterial infections, with winter ulcer disease caused by *Moritella viscosa* being particularly problematic in Northern Norway. The skin, composed of layers such as mucus, epidermis, dermis, and hypodermis, acts as the first line of defense against environmental changes, physical damage, and infections. Understanding the responses triggered in these skin layers is crucial for developing effective strategies to enhance fish health and resilience against environmental stressors and infections.

**Materials and methods**

Direct exposure of scale explants (SE) from Atlantic salmon of various sizes to marine commensal bacteria of the skin, as well as the skin pathogens *M. viscosa* and *Tenacibaculum finnmarkense*, has been utilized to investigate initial bacterial-skin interaction responses. Additionally, a full commercial-scale Atlantic salmon feeding trial was conducted under Arctic conditions, testing different levels and sources of dietary lipids. The fish were fed one of three diets: 7.5% or 10% of the fatty acids (FA) as EPA and DHA from fish oil, or 10% of the FA as EPA and DHA from a combination of 5% fish oil and 5% algae oil, each in triplicate net-pens. All three diets were designed to meet the known nutritional requirements of Atlantic salmon. Data from the end of the production cycle at slaughter will be presented to demonstrate dietary effects on skin, including SE to examine lipid impacts on *M. viscosa* responses. Gene expression analysis was performed using microarray, cell morphology, stress and migration capacity was assessed using imaging and immunocytochemical detection, and skin health and characteristics evaluated for a more comprehensive understanding of the initial bacterial-skin interaction responses.

**Results and conclusion**

The SE model is a promising tool for studying skin responses. Compared to other tissue types, it has demonstrated strong immune activity with expected high expression of skin-specific genes. Direct exposure of SE to the skin pathogens *M. viscosa* and *T. finnmarkense*, consistently reproduces a panel of immune genes in Atlantic salmon across various growth stages. These responses are regulated in a dose-dependent manner. The prevalence of genes related to signaling and communication suggests that salmon scales act as sentinels, mobilizing immune responses upon bacterial encounters. Although variations are observed between bacterial species, a consistent gene set with high expression responding to bacteria has been identified. The SE model also revealed faster migration of skin cells (keratocytes), facilitating wound closure, and stimulation of genes for epithelial cell cross-linking in higher dietary lipid groups of the fish trial. Exposure to *M. viscosa* further enhanced immune responses in SE with higher lipid levels. This finding corroborated trial observations, where the low dietary lipid group exhibited higher mortality due to winter ulcer disease. Overall, the SE model has proven effective in advancing research on host-pathogen skin interactions.

# A DIATOM-BASED BIOASSAY FOR ENVIRONMENTAL CONTAMINANTS: IMPLICATIONS FOR AQUATIC ECOSYSTEMS AND AQUACULTURE WATER QUALITY

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**Introduction**

Aquatic ecosystems are highly sensitive to environmental changes and are increasingly affected by a variety of pollutants including heavy metals and nanomaterials such as graphene oxide (GO), especially in regions affected by industrial or military activities. Due to their persistence and potential for bioaccumulation, these pollutants pose a serious risk to ecosystem function, biodiversity, and water quality, with implications for aquaculture and food safety. Among aquatic organisms, primary producers such as diatoms play an important ecological role in water bodies as they form the basis of the food web and drive oxygen production, and nutrient cycling. Due to their ecological importance and high sensitivity to pollutants, diatoms are widely used as effective bioindicators for the assessment of environmental hazards such as heavy metal and nanoparticle contamination.

This study evaluated the potential use of the freshwater diatom *Nitzschia palea* as a model organism in diatom-based bioassays for monitoring water quality, particularly in aquaculture systems and in the ecotoxicological assessment of contaminated or conflict-affected freshwater environments.

**Materials and methods**

A short-term (96 hours) bioassay was performed following the OECD guidelines for freshwater algal growth inhibition tests. *N. palea* was exposed under laboratory conditions to six concentrations of a metal mixture (MIX: Ni (II), Zn (II), Cr (III), Cu (II)) and graphene oxide (GO) individually, as well as in combination (MIX+GO). The concentrations were based on EU maximum permissible concentrations (MPC) for inland waters and were adjusted across a gradient from 0.1× to 80× MPC. Diatom growth rate and morphological changes were determined using a light microscope. Photosynthetic pigment levels (chlorophyll-a and carotenoids) were evaluated spectrophotometrically according to Zavřel et al. (2015). Lipid peroxidation was assessed in terms of malondialdehyde (MDA) content, according to Al-Rashed et al. (2016).

**Results**

Significant inhibition of the growth of *N. palea* and morphological changes, such as valve deformation, were induced by high metal concentrations (≥20 times higher than MPC) and MIX+GO co-exposure (>10 mg/L). Chlorophyll-a and carotenoids content showed a similar trend to the growth rate. In contrast, exposure to GO alone had no significant effect on the species growth, and *N. palea* was observed to utilise GO as a substrate. However, changes in pigment content were observed, with a slight increase at 10 mg/L, indicating a possible compensatory response to light limitation or early oxidative stress, followed by a sharp decrease at ≥20 mg/L. This decrease indicates GO induced photosynthetic inhibition, likely due to a shading effect, marking this concentration as a threshold for photosynthesis.

No significant changes in MDA levels, a marker of lipid peroxidation, were observed at low concentrations (≤10 mg/L) of MIX, GO, and MIX+GO, indicating minimal oxidative stress. However, at higher concentrations (≥20 mg/L), MDA levels decreased significantly, which could reflect either severe cell damage that suppressed lipid peroxidation or activation of antioxidant defence mechanisms.

**Conclusions**

Overall, *N. palea* demonstrated measurable physiological and morphological responses to metals and GO exposure, supporting its use in diatom-based bioassays for water quality monitoring. Such bioassays can provide valuable early-warning indicators of environmental pollution. Their application is particularly important for monitoring water quality in aquaculture systems and in conflict-affected freshwater areas, where maintaining ecosystem health is crucial for ensuring sustainable food production and protecting biodiversity.

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**Acknowledgement**
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# ENHANCING TRUST AND SUSTAINABILITY IN MALTESE SEAFOOD SUPPLY CHAINS

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**Introduction**

The FishEUTrust project, funded by the EU Horizon Europe programme, aims to improve trust, transparency, and sustainability in European seafood supply chains. The Malta Living Lab focuses on social innovation and stakeholder engagement. It acts as a collaborative platform uniting industry, government, researchers, civil society, and the public to promote sustainable seafood practices and ocean literacy in Malta, supporting the development of a circular seafood economy.

**Materials and Methods**

The Living Lab has actively engaged over 400 seafood stakeholders through a dynamic mapping process, which was further refined via collaborative workshops, including sister project Sea2See. To broaden public awareness and foster community involvement, the Lab participated in prominent Maltese events such as “FishFest,” “Science in the City,” and “Science in the Citadel,” all supported by European Maritime Day. Recognizing the importance of youth engagement, the Lab organized hackathons that inspire innovation and promote environmental stewardship among younger generations. Industry-focused workshops addressed critical topics such as sustainable sourcing and the valorisation of fish side-streams, facilitating a co-creative environment between stakeholders. Beyond local efforts, the Living Lab contributes to global ocean sustainability by participating in international initiatives, including a UN Ocean Decade event, and supports education through involvement in the Aqua Nor 2023 Student Camp. The Lab’s impact on ocean literacy is further exemplified by its leader’s recognition as a Youth4Ocean Forum Young Ocean Advocate, underscoring the Lab’s commitment to nurturing informed and active ocean stewards.

**Results**

The Malta Living Lab has become a key hub for social innovation and ocean literacy in Malta’s seafood sector. Collaborative workshops with Sea2See improved stakeholder engagement and produced a shared action plan, strengthening outreach. Public events successfully increased awareness of sustainable fisheries. Participation in European Ocean Days and UN Ocean Decade conferences enabled sharing of project outcomes and best practices with European partners, boosting Malta’s role in marine sustainability discussions.Youth hackathons and initiatives like Aqua Nor 2023 inspired creative marine conservation solutions and emphasized traceability and consumer trust. Industry workshops fostered collaboration on circular economy practices. International recognition, including the Youth4Ocean Forum award, has strengthened Malta’s voice in European and global marine sustainability. Plans to form a seafood cluster aim to sustain these collaborative efforts beyond FishEUTrust, supporting ongoing innovation and sustainable development in Maltese aquaculture and fisheries.

# TARGETING *VIBRIO* IN LIVE FEEDS: BACTERIOPHAGE COCKTAILS AS A PREVENTIVE TOOL IN MARINE HATCHERY SYSTEMS

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**Introduction**

Disease outbreaks in marine aquaculture continue to pose major challenges, particularly during early larval stages when fish are highly susceptible to opportunistic infections and their immune system is still immature. Fish larvae depend exclusively on live feeds such as Rotifers and Artemia, which often serve as reservoirs of pathogenic bacteria—especially members of the *Vibrio* genus. Given the global effort to reduce antibiotic use, bacteriophages—viruses that exclusively infect and lyse bacteria—have gained attention as a highly specific, eco-friendly alternative for bacterial control in aquaculture environments.

In this study, we have tested the efficacy of fully characterized phages against known pathogenic vibrios but also against randomly selected vibrios from the live feed section of a research fish hatchery. The goal was to identify optimal phage candidates and cocktail combinations for further application in hatchery systems as a preventive intervention against fish infections by member of the *Vibrio* genus.

**Materials and Methods**

Water samples were collected from Artemia and Rotifer culture tanks at the hatchery of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC). Bacterial strains were isolated on thiosulfate-citrate-bile salts-sucrose (TCBS) agar, a selective medium for *Vibrio* species. Seventeen distinct isolates were selected based on colony morphology and preliminary biochemical characteristics for subsequent testing. To enhance the diversity and pathogenic relevance of this bacterial panel, four previously characterized *Vibrio* strains from the IMBBC lab collection were also included; *Vibrio harveyi* VH2, *V. alginolyticus* V1, *V. anguillarum* VAK, and *V. harveyi* KEF80, all of which linked to disease outbreaks in fish hatcheries.

A panel of bacteriophages from IMBBC lab collection was screened against the full bacterial set. Phages had been previously isolated from marine environments and propagated on either *Vibrio alginolyticus* or *V. harveyi.* Five phages (Figure 1), Vibrio phage Virtus (Droubogiannis and Katharios, 2022), Vibrio phage ELK1, Vibrio phage Artemius (Droubogiannis et al., 2022), ST2 (Kalatzis et al., 2016), and Vibrio phage FG which is a novel phage, were selected based on their plaque clarity, host range, and genomic profiles. Whole genome sequencing confirmed the absence of undesirable genes (e.g., lysogeny markers, toxins).

Each phage was evaluated in *in vitro* killing assays against the bacterial panel. Cultures were incubated with phage suspensions in liquid media, and optical density ($OD_{600}$) was monitored over time to generate detailed lysis curves. Phage cocktails were then formulated using two or three phages, targeting maximal bacterial coverage while aiming to reduce the likelihood of resistance emergence. The same *in vitro lysis* assays were applied to test cocktail performance.

**Results and Discussion**

The results highlighted clear variability in host susceptibility. Certain phages, such as Virtus, ST2 and FG, showed strong, rapid lysis and a relative broader host range and were selected for further downstream applications. Several cocktails demonstrated improved bacterial clearance compared to individual phages, suggesting additive or synergistic interactions (Figure 2).

Collectively, our results demonstrate the feasibility of isolating representative bacteria from live feed systems and effectively targeting them with well-characterized phages. These *in vitro* findings establish a strong foundation for the development of phage-based disinfection protocols tailored to hatchery settings. Our findings provide meaningful insights into phage-host interactions, cocktail optimization, and the potential for routine phage supplementation of live feeds to reduce microbial risks during early fish development. Phage therapy could be used to selectively exclude unwanted bacteria from fish hatcheries leaving beneficial bacteria unaffected by a targeted modulation of the microbiome.

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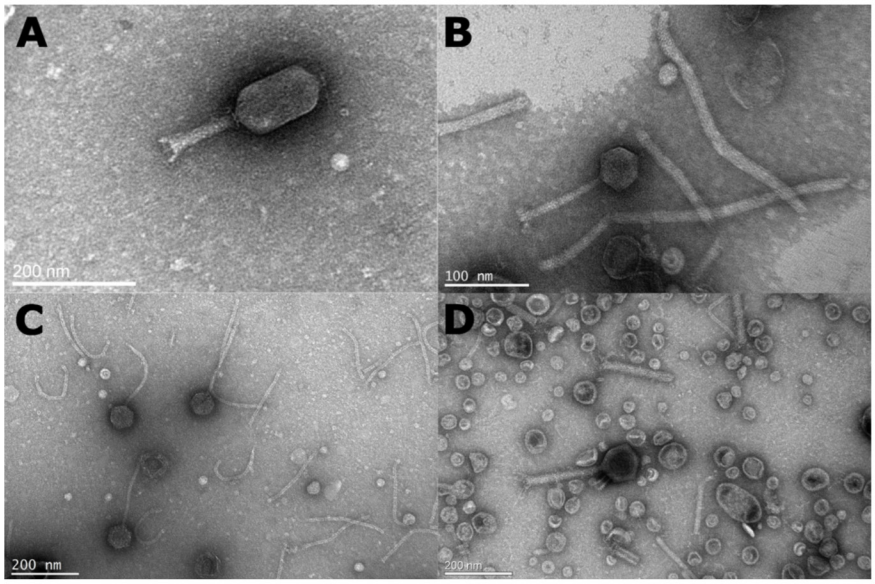


**Figure 1.** Transmission electron microscopy pictures of phage St2 (A), Artemius (B), Virtus (C) and FG (D).

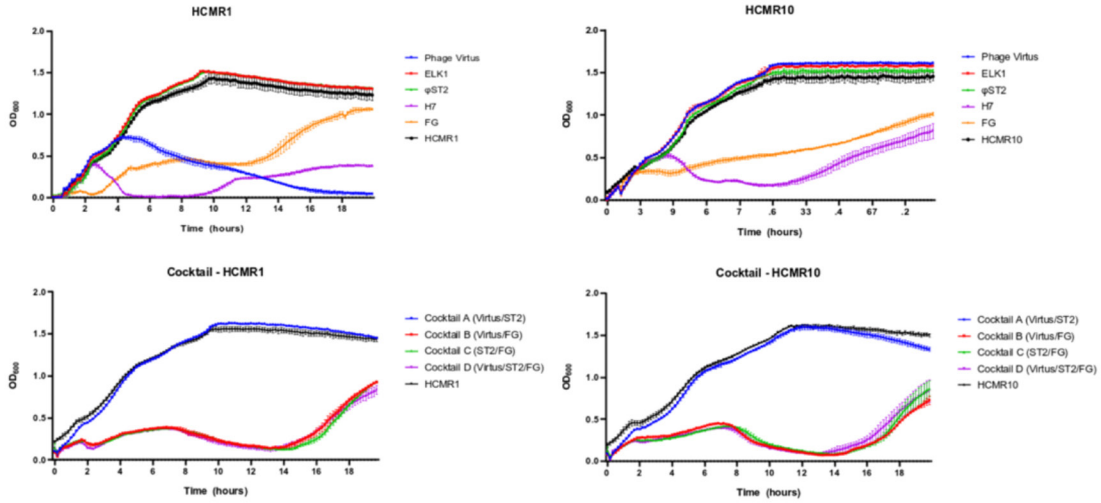


**Figure 2.** In vitro lysis effect of single phages (upper graphs) compared to phage cocktails (lower graphs) over time in two randomly selected vibrios from the HCMR's live feeds.

The study has been funded in part by the European Union's Horizon Europe Research and Innovation Programme under grant agreement 101084204 Cure4Aqua.

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# LIPID DROPLET DISTRIBUTION IN ATLANTIC SALMON *(Salmo salar L.)* OOCYTES IS RELATED TO THE FERTILISATION AND DEVELOPMENTAL OUTCOME

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## Introduction

Variation in oocyte quality is one of the main constraints for a sustainable production in aquaculture industry. Enhanced knowledge on oocyte quality criteria would contribute to a better reproductive success in the aquaculture industry (Kjørsvik, Mangor-Jensen, & Holmefjord, 1990).Several studies have investigated lipid droplet distribution in various fish species, rainbow trout (Ciereszko, Wojtczak, Dietrich, Kuźmiński, & Dobosz, 2009), brown trout (Mansour, Lahnsteiner, & Patzner, 2007) and Arctic char (Mansour, Lahnsteiner, McNiven, & Richardson, 2008). The current study is based on the area size of the lipid droplet distribution and the study confirms that there is a significant correlation between subgroups and fertilisation outcome.

## Materials & Methods

From 16 females, oocytes from 8 females were selected with fertilisation rates >90 % (high quality) and oocytes from 8 other with fertilisation rates between 85 and 0 % (low quality). A fertilisation assay was performed to obtain fertilisation rate, eyed embryo success and hatching success. Oocytes were picked randomly from each female and imaged under a MZ1500 stereoscopic zoom microscope with the 1×- lens (High Resolution plan Apo 1×) coupled with 10× eyepieces (Nikon Instruments Inc., New York, USA). The Nikon NIS-Elements Research (version 3) software was used to capture images. The images were taken from two angles by first capturing the coalesced lipid area, if there was any, and then turning the oocyte 180◦ for capturing a second image. The area size of the lipid coalescence was measured using free hand tool in Image J software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA) which is a free, opensource image processing program. All the images were adjusted to the scales (1DIV = 0.01 mm) and processed.

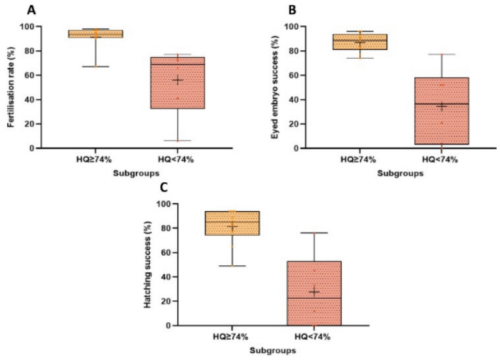


*Figure 1. Box and whisker plots presenting differences in subgroups with lipid distribution patterns based on fertilisation outcome in Atlantic salmon (Salmo salar L).*

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**Results & Discussion**

Based on the distribution of lipid droplets, five categories of oocytes were classified. Category 1 (C1), lipid droplets were distributed evenly while C2 had one or two small lipid globules starting to coalesce in a smaller area with average area size and standard deviation of 0.34 mm2 ± 0.15. In C3, more lipid droplets had started coalescing and had spread into a greater area with an average area size of 1.0 mm2 ± 0.25. C4 had the largest areas with coalesced lipid droplets which emphasized the area with darker orange colour with an average area size of 2.43 mm2 ± 0.76. In C5, the lipid droplets were almost all coalesced and formed one large lipid globule with an average area size of 8.53 mm2 ± 2.38, which in hatcheries are known as over-ripe. Average area size of an oocyte is approximately 12 $mm^2$ ($n$ = 50).

For further analysis, the categories 1,2,3 were included in subgroup HQ when the number of oocytes (%) in HQ were above or equal to 74 % and 4 and 5 were included in subgroup LQ when the number of oocytes (%) in HQ were below 74 %. To investigate the association between lipid droplet distribution and fertilisation outcome, females were sorted based on the distribution of oocytes within the HQ subgroup. Ten of the females had oocytes with higher or equal to 74 % (range 74–100 %) from subgroup HQ and six females had oocytes with less than 74 % (range 5–64 %) from HQ. According to the results presented in Fig. 2A, a higher fertilisation rate (≥90 %) was obtained when there were more oocytes (≥74 %) from subgroup HQ present in the oocyte batch. The fertilisation rate was significantly lower (<78 %) when less oocytes (≤64 %) from subgroup HQ ≥ 74 % were present in the oocyte batch. A similar pattern is shown in Fig. 2B and C between subgroups and subsequent eyed embryo success and hatching success, which resulted in a higher eyed embryo success (>70 %) and hatching success (>65 %) in the presence of more oocytes from subgroup HQ ≥ 74 %.

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# A REVIEW OF THE EFFECTS OF KRILL MEAL ON KEY NON-SALMONID FISH SPECIES IN AQUACULTURE: INSIGHTS FROM 22 NUTRITIONAL STUDIES (1980S-PRESENT)

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With the growing global demand for fish as a protein source, there's increasing pressure to find sustainable alternatives to traditional aquafeed ingredients like fishmeal (FM) and fish oil (FO), which are facing reduced availability due to environmental and economic constraints. Plant-based substitutes help ease the burden on marine ecosystems but come with challenges such as anti-nutritional factors, low levels of essential omega-3 fatty acids (EPA and DHA), limited amino acid profiles, and reduced palatability—ultimately affecting fish growth, health, and product quality. Moreover, only a few crops like soybean, rapeseed, corn, and wheat are commonly used in aquafeeds, and their availability is influenced by climate change and global socio-economic factors. These plant-based ingredients also compete with human food, livestock, and pet food markets, driving up prices and limiting supply for aquaculture. Novel feed ingredients like insect meals, single-cell proteins, and fish co-products are being explored but are still limited by regulatory, cost, and scalability issues. Antarctic krill (*Euphausia superba*) stands out as a promising low-trophic alternative with minimal environmental impact, sustainable sourcing as it's the most sustainable fishery, aligning with industry standards and consumer demands for eco-friendly products. Moreover, since krill is not consumed by humans, there is no competition between its use for food and feed. Further krill-derived products mainly krill meal (KM) are rich sources of nutrients such as highly digestible proteins and lipids, phospholipids, astaxanthin, and phospholipid-bound omega-3s (EPA and DHA), which are more bioavailable than those in traditional sources and micronutrients such as organic minerals and vitamins. Further, commercially available KM is supported by extensive scientific evidence demonstrating its benefits in various fish species when included in feeds. This review consolidates current knowledge based on 22 nutritional studies from the 1980s to the present on the benefits of KM for the most important non-salmonid fish species to aquaculture, including Gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), Nile tilapia (*Oreochromis niloticus*), red seabream (*Pagrus major*), Olive flounder (*Paralichthys olivaceus*), and yellowtail (*Seriola quinqueradiata*) (Figure. 1). In addition, this review addresses the possible future perspectives on the application of krill meal in aquaculture feeds. Finally, the review addresses the key challenge of KM's high cost, highlighting the need for comprehensive economic evaluations to assess its cost-effectiveness compared to both conventional and novel feed ingredients.

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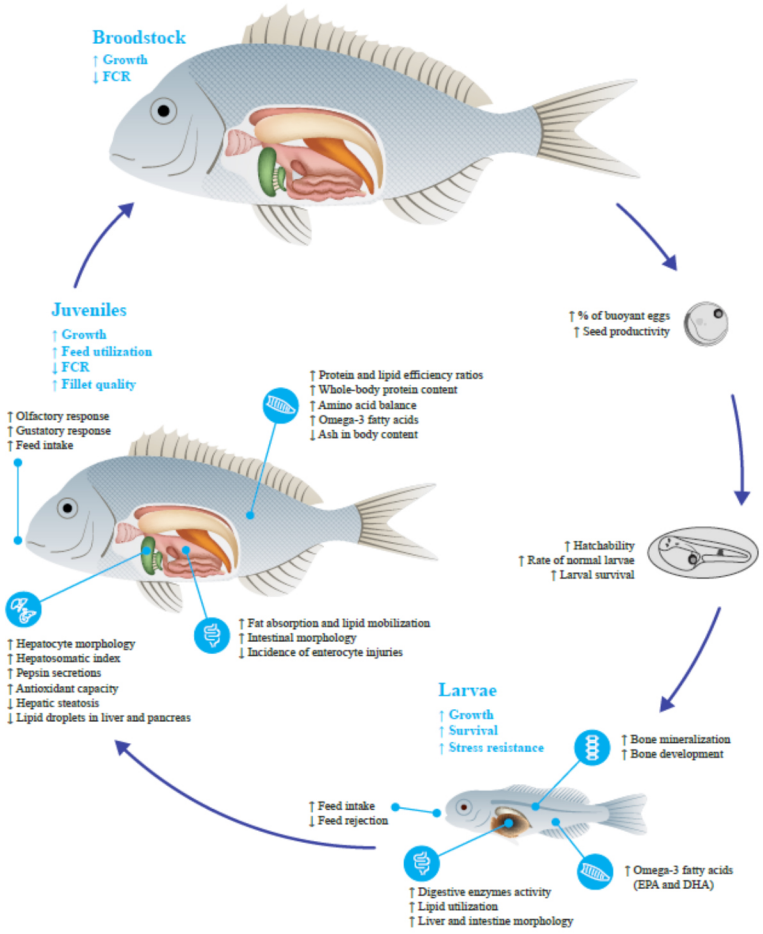


**Figure**: Main effects of krill products on farmed non-salmonid fish, as illustrated on gilthead seabream development stages as a example. In blue, main general effects at each development stage. Effects on specific organs are indicated close to the icon representing the organ(s). FCR, feed conversion ratio.

# A NOVEL NETWORK APPROACH TO ESTIMATE THE ECONOMIC BURDEN OF LISTED AQUATIC ANIMAL DISEASE OUTBREAKS AND CONTROL MEASURES

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**Introduction**

Serious aquatic diseases, once introduced into a disease-free country or zone, can rapidly spread between aquaculture sites and cause substantial economic losses due to high mortality rates among cultured species. Epidemics can be managed using control measures such as contact tracing, movement restrictions, culling, disinfection, fallowing and surveillance, mitigating further spread and, in turn, reducing overall economic burden. However, controls also cause economic cost to the industry and to those responsible for enforcing them. Understanding the cost-benefit of control measures is, therefore, necessary for evaluating and justifying their merit. Mathematical modelling frameworks offer value in filling this gap. For example, AquaNet-Mod, an aquaculture network model, has previously been applied to simulate viral haemorrhagic septicaemia virus (VHSv) epidemics and controls across the salmonid industry in England and Wales using real-world connectivity data (Guilder et al. 2023). Here, this study extends the model framework by incorporating economic burden data incurred by each aquaculture site due to VHSv infection and control and extrapolate it at a national level. This innovative approach aims to directly strengthen evidence-based decision-making for epidemiological and cost-efficient disease control strategies.

**Materials and Methods**

Economic data that represents expected site level burden during VHSv epidemics was collected using the typical farm approach (Lasner *et al*., 2020). Each site within the network was classified as a certain type of typical aquaculture site based on real world data on culture method and levels of production, such as hatcheries, re-stockers and table producers. Farmers representative of all typical farms took part in an on-site survey to identify changes to monthly revenue and operating costs expected under different disease and control states and any additional one-off costs. The resulting estimations of economic burden at the typical farm level were incorporated into AquaNet-Mod simulations. This was accomplished by applying the relevant costs to each site based on the duration it spent in an infection or control state. Epidemic simulations were run under the current English and Welsh control strategy, and with the removal of contact tracing and all controls. During the simulations, the total national economic burden was calculated by 1) associating the disease and control state of each site with relevant typical farm costs and 2) cumulating these costs across the entire network. This process allows for the estimation of the total national burden based on the number of sites impacted and what those impacts were.

**Results**

Implementing disease controls significantly reduced the number of infected sites and associated costs (Figure 1). In the absence of disease controls, there was a significantly greater chance of larger, long-lasting epidemics with greater economic burdens (Figure 1C). Control measures were shown to decrease the total number of infected sites (Figure 1A), which, in turn, reduced the overall epidemic cost by minimising the need for sites to be culled, disinfected and undergo fallowing, which were the greatest individual site level costs reported during the typical farm surveys. Additionally, the simulations demonstrated the effectiveness of contact tracing in reducing the likelihood of larger and costly epidemics (Figure 1B). Contact tracing had low implementation costs to the industry, whilst reducing the likelihood of the largest and most costly epidemics by effectively reducing spread between sites. Furthermore, the merit of the combination of all controls was shown during the *no controls* scenario, where the likelihood of larger, more costly epidemics increased the average total epidemic cost 12-fold.

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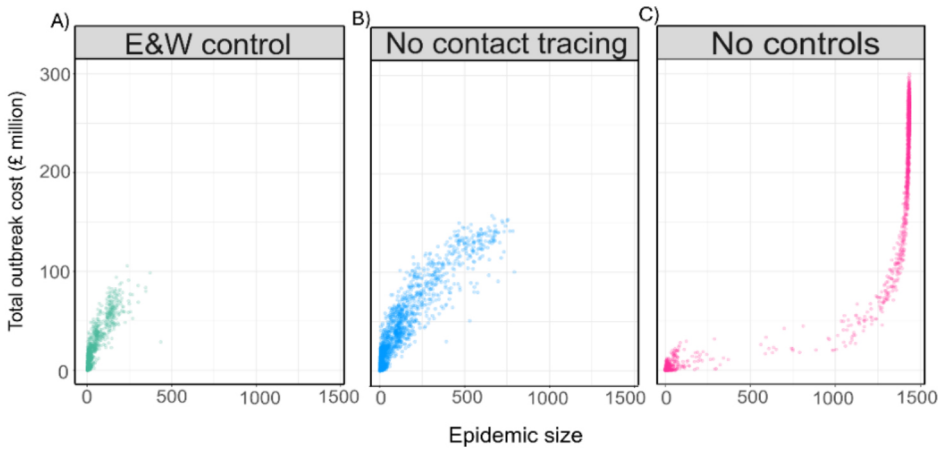


Fig 1. Simulations of viral hemorrhagic septicemia virus (VHSv) epidemics across the England and Wales salmonid sector in terms of epidemic size (number of sites infected) and the associated economic cost to industry. A) Cost and size of epidemics under current control protocols. B) Cost and size of epidemics with contact tracing removed. C) Cost and size of epidemics with all controls removed.

**Discussion**

To our knowledge, this study represents the first assessment of the impact of disease control on national economic burden of epidemics based on the combined use of network modelling and site-level economic assessment methodologies. The integration of estimated site-level costs collected via typical farm surveys into AquaNet-Mod provides key novel insights into the economic impact of VHSv introductions into the salmonid sector in England and Wales. The method generated clear evidence on the merit of control measures in reducing epidemic size and associated costs, providing valuable information to inform optimal control strategies. For instance, if VHSv incursions were to occur within the England and Wales salmonid sector, prioritising contact tracing during the early stages of an outbreak is expected to significantly reduce the likelihood of a widespread epidemics that jeopardise the entire industry. This integrated approach, combining the expertise of epidemiologists, modellers and economists, has demonstrated a flexible and adaptable framework with the potential for application to other diseases and aquaculture sectors.

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# DIETARY YEAST HYDROLYSATE IMPROVED THE PERFORMANCE OF JUVENILE AND GROWER ASIAN SEABASS (*Lates calcarifer*)

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## Introduction

Asian seabass (*Lates calcarifer*) is an omnivorous, euryhaline fish with commercial importance especially in Southeast Asian aquaculture. The performance of Asian seabass is often decreased by pathogenic bacteria, such as *Vibrio* spp. or *Aeromonas* spp. Dietary strategies to improve fish performance are needed. The present study investigated the effects of dietary *Saccharomyces* yeast hydrolysate (YH) on the performance of juvenile and grower stage Asian seabass which were grown in earth ponds in Thailand.

## Materials and methods

Two experiments were conducted: E1 for juvenile fish, with a mean body weight 13g on day 1, and E2 for the grower stage, with a mean body weight 270 g on day 1. Both experiments were carried out in net cages, in earth ponds of 0-3 ppt saline water, which was aerated to maintain DO>5 mg/l. Commercial-type feed for Asian seabass was amended with different levels of YH (Progut® Extra, Hankkija Oy, Finland) for the dietary treatments. The set-up for E1 was 30 fish/cage, density of 15 fish/m$^3$, 3 treatments with YH levels of 0.0, 0.15, or 0.30%, 4 pens/tr, and a trial period of 8 weeks. In E2 there were 10 fish/cage, density of 5 fish/m$^3$, 4 treatments with YH levels of 0.0, 0.10, 0.15, or 0.20%, 5 replicates/tr., and trial period of 12 weeks. In both experiments, the fish were fed three times a day at 3-5% of body weight. Remaining feed was siphoned out, dried and weighed for calculating feed intake. Fish weight gain and feed conversion ratio (FCR) were recorded every two weeks. On the final day, blood samples were collected for the analysis of white and red blood cell counts, hemoglobin, hematocrit, immunoglobulin M (IgM), lysozyme activity, and glutathione. Foregut villus length was measured from histological samples. In E2, superoxide dismutase (SOD) activity was measured from blood samples, and the carcass composition was determined. Study design was completely randomized in both experiments, and one-way ANOVA and Duncan's Multiple Range Test were used for data analysis. Limit for statistical significance was set to $p<0.05$.

## Results

In E1 (Table 1), dietary YH significantly increased average daily gain, fish weight on the final day, and feed intake. Feed conversion and mortality were similar in all treatments. Hematocrit was increased by YH, while YH significantly decreased the red and white blood cell counts. The treatments did not significantly affect serum protein concentration, immunoglobulin M, glycogen, lysosome activity, glutathione, or foregut villus length.

In E2 (Table 2), YH significantly and dose-dependently improved the average daily gain, and also increased fish weight at 12 week. Feed conversion was significantly improved by dietary YH amendment. Mortality was 0% in all treatments throughout the experiment. Hematocrit, serum protein concentration, mean villus length, lysozyme activity and glutathione were all unaffected by the dietary treatments. The SOD activity tended to be higher for YH-treated groups than the control group ($p=0.068$). Carcass composition and foregut villus length were not affected by the dietary treatments.

## Discussion and conclusions

In conclusion, dietary YH supplementation had a positive effect on the weight gain and final weight of juvenile and grower Asian seabass. Feed conversion was improved by dietary YH in the grower age group. Dietary YH may be a strategy for improving the performance of Asian seabass. Immunological or physiological mechanisms behind the observed performance effects remained inconclusive, and open for further studies.

*(Continued on next page)*

**Table 1.** Effect of the dietary treatments on parameters in Experiment 1.

| Item | T1[1] | T2[2] | T3[3] | *p*-value |
|---|---|---|---|---|
| **Performance** | | | | |
| Fish weight, week 8 (g) | 52.35[b] | 66.27[a] | 67.18[a] | 0.043 |
| ADG[4], week 0-8 (g) | 0.70[b] | 0.95[a] | 0.96[a] | 0.039 |
| Feed consumption (g/fish) | 53.57[b] | 61.66[ab] | 75.85[a] | 0.045 |
| FCR[5] (g/g) | 1.37 | 1.19 | 1.40 | 0.126 |
| **Analyses, week 8** | | | | |
| RBC[6] count (*$10^5$ cell/ml) | 6.90[a] | 4.15[b] | 4.13[b] | 0.001 |
| WBC[7] count (*103 cell/ml) | 78.3[a] | 50.0[ab] | 25.0[b] | 0.035 |
| Hematocrit (%) | 16.0[b] | 16.0[b] | 24.0[a] | 0.002 |
| Hemoglobin (g/l) | 4.91 | 5.02 | 6.82 | 0.075 |

[1]YH 0.0%, [2]YH 0.15%, [3]YH 0.3%, [4]average daily gain, [5]feed conversion ratio, [6]red blood cell, [7]white blood cell, Different letters in the same row indicate significant differences between treatments ($p<0.05$).

**Table 2.** Effect of the dietary treatments on parameters in Experiment 2.

| Item | Dietary treatments | | | | |
|---|---|---|---|---|---|
| | T1[1] | T2[2] | T3[3] | T4[4] | *p*-value |
| **Performance** | | | | | |
| Fish weight, week 12 (g) | 550.1[b] | 617.5[a] | 626.3[a] | 639.8[a] | 0.010 |
| ADG[5], week 0-12 (g) | 3.39[b] | 4.09[a] | 4.28[a] | 4.37[a] | 0.017 |
| Feed consumption 0-12 (g/fish) | 791 | 791 | 791 | 791 | 1.000 |
| FCR[6], week 0-8, (g/g) | 2.78[a] | 2.21[b] | 1.87[b] | 1.91[b] | 0.013 |
| FCR[6], week 0-10, (g/g) | 2.85[a] | 2.20[b] | 2.02[b] | 2.02[b] | 0.001 |
| FCR[6], week 0-12, (g/g) | 2.86[a] | 2.31[b] | 2.21[b] | 2.18[b] | 0.014 |
| **Analyses, week 12** | | | | | |
| RBC[7] count (*$10^5$ cell/ml) | 7.78 | 7.32 | 7.76 | 7.52 | 0.737 |
| WBC[8] count (*$10^4$ cell/ml) | 1.70 | 1.70 | 1.80 | 2.40 | 0.712 |
| Hematocrit (%) | 33.0 | 36.4 | 36.6 | 36.2 | 0.403 |
| Hemoglobin (g/l) | 4.76[b] | 4.40[b] | 4.46[b] | 5.35[a] | 0.014 |
| SOD[9] activity (U/ml) | 4.99 | 7.76 | 8.79 | 7.83 | 0.068 |

[1]YH 0.0%, [2]YH 0.10%, [3]YH 0.15%, [4]YH 0.2%, [5]average daily gain, [6]feed conversion ratio, [7]red blood cell, [8]white blood cell, [9]superoxide dismutase, [a, b, c] Different letters in the same row indicate significant differences between treatments ($p<0.05$).

# EVALUATION OF FERMENTED SUGAR BEET PULP AS A POTENTIAL INGREDIENT IN THE FEED OF *Labeo rohita* (ROHU)

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A feeding trial was conducted to evaluate fermented sugar beet pulp (FSBP) as a potential ingredient in the feed for *Labeo rohita* (Rohu). *L. rohita* juveniles (average initial weight 15 g) were selected as experimental fish for this study. The experiment was carried out in 21 glass aquariums. The trial was divided into seven (7) treatments each having three replicates. Seven isonitrogenous diets were formulated by incorporating raw and fermented sugar beet pulp (SBP) at 0% (Control), 7%, 14%, and 21% levels. Baker's yeast waste was used for the fermentation of SBP. After the feeding trial, fish were randomly sampled from each treatment for growth monitoring, body composition, blood and serum biochemical analysis, digestive and antioxidant enzymes. In terms of growth performance of *L. rohita*, diet T6 containing 21% FSBP resulted in a significantly ($P<0.05$) higher growth. The overall performance of *L. rohita* fed fermented FSBP incorporated diets was better in comparison to those fed with RSBP incorporated diets and control. The activities of anti-oxidant and digestive enzymes were observed significantly ($P<0.05$) higher in fish fed with 21% FSBP (T6). The maximum deposition of protein in the carcass was recorded in fish fed with 21% FSBP diet (T6). The supplementation of FSBP in the diets significantly affected the haematological status of fish ($P<0.05$). To assess liver health, serum enzymes (AST, ALT, and ALP), lactate dehydrogenase, cholesterol, triglycerides, and total proteins the fermented sugar beet pulp in the feed positively impacted these health markers. In conclusion, it has been observed that the supplementation of FSBP up to 21% in *L. rohita* diet, effectively improved health parameters without compromising growth performance, haemato-biochemistry, and liver functionality.

# EVALUATION OF DIETARY MICELLE FORM OF COENZYME Q10 IN DIET FOR OLIVE FLOUNDER, *Paralichthys olivaceus*

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## Introduction

Coenzyme Q10 (CoQ10), known as ubiquinone, is a coenzyme that functions as an electron transporter in the ATP production. CoQ10 acts as an antioxidant with sparing effect for vitamin C and E. Micelle structures possess an interface between hydrophilic and hydrophobic area, which can protect feed additives and facilitate their absorption in the body. Olive flounder (*P. olivaceus*) is the main aquaculture species in South Korea. This study was conducted to evaluate dietary CoQ10 and micelle form CoQ10 (MCoQ10) supplementations in diet for olive flounder feed.

## Materials and methods

A basal diet (Con) was formulated to contain 600 g/kg fish meal and 120 g/kg soybean meal as main protein sources. Seven other diets were prepared adding 20 and 40 mg CoQ10/kg (CQ2 and CQ4, respectively), and 10, 20, 40, 80 and 160 mg MCoQ10/kg (MQ1, MQ2, MQ4, MQ8 and MQ16, respectively). Experimental fish (initial mean body weight: 24.1 g) were randomly distributed into 24 tanks (300 L), with 30 fish per tank in triplicates, and fed the diets twice daily for a 66-day feeding trial. At the end of feeding trial, all fish were individually counted. To test the disease resistance against *Edwardsiella tarda*, the remaining fish were re-distributed into 24 acrylic tanks (120 L) with 12 fish per tank in triplicates. Each fish was injected with 100 μL of $1.0\times10^7$ CFU/mL *E. tarda* suspension ($5.0\times10^4$ CFU/fish). Mortality was monitored at 6-h intervals for 8 days (186 h).

## Results

The final mean body weight and protein efficiency ratio were significantly higher in MQ4 and MQ8 groups compared to those in Con group. Ig level and lysozyme activity were significantly enhanced in all CQ and MQ groups. Anti-protease and phagocytic activities in MQ2, MQ4, MQ8 and MQ16 groups were significantly higher than in Con group. Antioxidant capacities (superoxide dismutase, glutathione peroxidase and catalase) were significantly improved in fish fed MQ2, MQ4 and MQ8 diets than in fish fed Con diet. Lipase activity was significantly increased in all CQ and MQ supplemented groups. Chymotrypsin and trypsin activities were significantly improved in CQ4, MQ8 and MQ16 groups compared to Con group. Intestine villi length, villi width and goblet cell counts were significantly increased in all CQ and MQ groups compared to Con group. Expression of inflammation-related genes were upregulated in CQ2, MQ1, MQ2, MQ4, MQ8 and MQ16 groups than in Con group. CoQ10 levels in liver and muscle were significantly higher in CQ4, MQ4, MQ8 and MQ16 groups than that in Con group. Vitamin C levels in liver and muscle were increased in MQ2 and MQ4 groups. Vitamin E levels in liver and muscle were the highest in MQ4 group. For intestinal microbiota, MQ8 and MQ16 groups showed significantly higher proportion of lactic acid bacteria, while all CQ and MQ groups exhibited lower proportions of *Vibrio* bacteria compared to Con group. Following the 186-h *E. tarda* challenge test, cumulative survival of all CQ and MQ groups were significantly higher than that of Con group.

## Conclusion

This study suggests that MCoQ10 supplementation could enhance growth performance, immune response, antioxidant capacity, intestinal microbiota and disease resistance against *E. tarda*. The optimal dietary supplementation level of MCoQ10 in diet for olive flounder is likely to be 40-80 mg/kg.

*(Continued on next page)*

Table 1. Growth performance and feed utilization of olive flounder *P. olivaceus* (initial mean body weight: 24.1 g) fed the diets for 66 days.

| Dietary treatments | FMBW[1] | SGR[2] | FCR[3] | PER[4] | Survival (%) |
|---|---|---|---|---|---|
| Con | 186±3$^{b}$ | 3.11±0.02 | 1.44±0.03 | 1.23±0.03$^{b}$ | 95.56±1.92 |
| CQ2 | 190±7$^{ab}$ | 3.14±0.05 | 1.41±0.02 | 1.25±0.02$^{ab}$ | 100±0 |
| CQ4 | 190±4$^{ab}$ | 3.14±0.04 | 1.41±0.05 | 1.25±0.04$^{ab}$ | 98.89±1.92 |
| MQ1 | 192±3$^{ab}$ | 3.14±0.04 | 1.42±0.05 | 1.27±0.04$^{ab}$ | 98.89±1.92 |
| MQ2 | 193±5$^{ab}$ | 3.14±0.05 | 1.44±0.03 | 1.24±0.02$^{ab}$ | 98.89±1.92 |
| MQ4 | 196±2$^{a}$ | 3.18±0.01 | 1.40±0.01 | 1.29±0.01$^{a}$ | 98.89±1.92 |
| MQ8 | 196±7$^{a}$ | 3.18±0.06 | 1.41±0.03 | 1.28±0.03$^{a}$ | 100±0 |
| MQ16 | 193±2$^{ab}$ | 3.13±0.02 | 1.44±0.01 | 1.25±0.01$^{ab}$ | 100±0 |

[1]FMBW, final mean body weight (g); [2]SGR, specific growth rate (%/day); [3]FCR, feed conversion ratio (feed intake/wet weight gain); [4]PER, protein efficiency ratio (wet weight gain/total protein given).

Table 2. Non-specific immune response and antioxidant capacity of olive flounder *P. olivaceus* fed the diets for 66 days.

| Dietary treatments | Ig[1] | Lysozyme[2] | NBT[3] | SOD[4] | GPx[5] | CAT[6] |
|---|---|---|---|---|---|---|
| Con | 14.8±0.77$^{c}$ | 5.37±0.39$^{c}$ | 0.46±0.02$^{d}$ | 63.5±1.2$^{c}$ | 116±11$^{c}$ | 71.1±2.0$^{b}$ |
| CQ2 | 19.5±0.40$^{b}$ | 6.65±0.09$^{b}$ | 0.58±0.02$^{bc}$ | 66.0±6.8$^{bc}$ | 135±12$^{abc}$ | 73.2±0.1$^{a}$ |
| CQ4 | 19.5±0.52$^{b}$ | 6.88±0.29$^{b}$ | 0.59±0.05$^{bc}$ | 66.8±4.8$^{ab}$ | 119±11$^{c}$ | 73.2±1.0$^{a}$ |
| MQ1 | 20.6±0.42$^{b}$ | 6.69±0.31$^{b}$ | 0.53±0.03$^{c}$$^{d}$ | 65.9±2.9$^{bc}$ | 125±20$^{bc}$ | 73.3±0.9$^{a}$ |
| MQ2 | 20.5±1.25$^{b}$ | 6.79±0.10$^{b}$ | 0.59±0.03$^{bc}$ | 73.0±1.8$^{ab}$ | 146±3$^{ab}$ | 73.5±1.0$^{a}$ |
| MQ4 | 22.8±0.88$^{a}$ | 6.75±0.64$^{b}$ | 0.60±0.04$^{a}$$^{b}$ | 73.3±3.6$^{a}$ | 144±12$^{ab}$ | 74.5±0.8$^{a}$ |
| MQ8 | 22.5±0.33$^{a}$ | 7.52±0.10$^{a}$ | 0.65±0.05$^{a}$$^{b}$ | 73.6±3.4$^{a}$ | 151±13$^{a}$ | 74.1±1.6$^{a}$ |
| MQ16 | 20.6±0.68$^{b}$ | 7.53±0.47$^{a}$ | 0.67±0.05$^{a}$ | 71.5±2.6$^{ab}$ | 139±5$^{abc}$ | 73.2±1.0$^{a}$ |

[1]Ig, immunoglobulin level (mg/mL); [2]Lysozyme activity (μg/mL); [3]NBT, nitro-blue tetrazolium activity (absorbance); [4]SOD, superoxide dismutase activity (% inhibition); [5]GPx, glutathione peroxidase activity (nmol/min/mL); [6]CAT, catalase activity (mU/mL).

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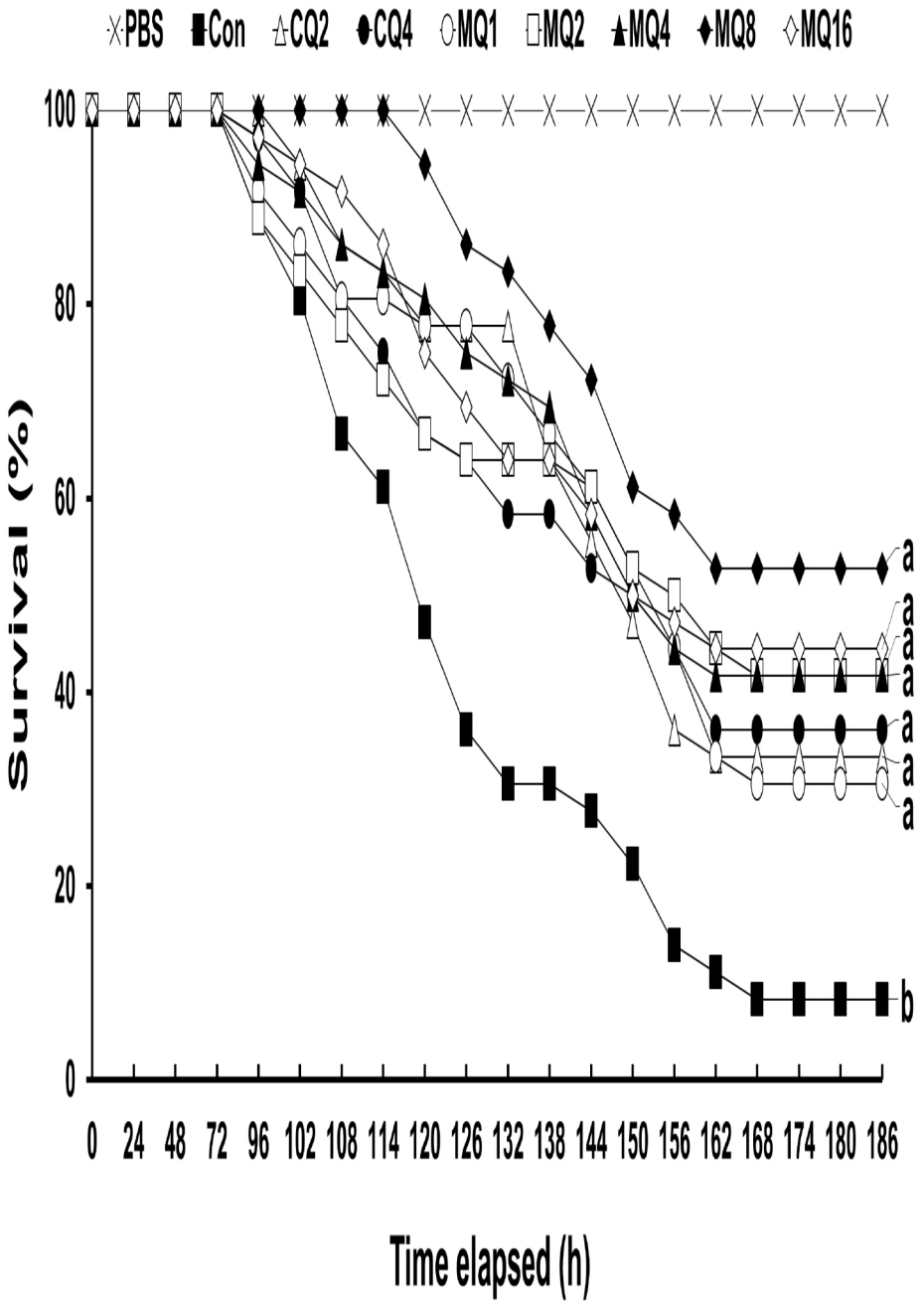


Figure 1. Survival of olive flounder *P. olivaceus* during the *E. tarda* challenge test. A 100 μL of $1.0\times10^7$ CFU/mL *E. tarda* suspension was injected into the fish ($5.0\times10^4$ CFU/fish).

# FISH OIL REPLACEMENT WITH A DHA-RICH *Schizochytrium* OIL ENHANCES GROWTH, IMMUNITY, ANTIOXIDANT CAPACITY AND DISEASE RESISTANCE OF PACIFIC WHITE SHRIMP *Penaeus vannamei*

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**Introduction**

Pacific white shrimp is the most economically valuable shrimp species in the world. Fish oil serves as a primary source of essential fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid which play critical roles in supporting growth, immune function, and disease resistance in shrimp. However, the decrease in fish stocks and the rise in global demand of fish oil are driving up feed prices and threatening marine ecosystems, underscoring the need for sustainable alternative resources. *Schizochytrium* sp. (SHC), a marine microalga rich in DHA, has emerged as a promising substitute for fish oil. SCH demonstrates environmental sustainability and ensures stable large-scale production and supply, with the distinct advantage of not competing with food sources intended for human consumption. This study was conducted to evaluate the effects of dietary fish oil replacement with SCH oil on the growth performance, innate immunity, antioxidant capacity, and disease resistance of *P. vannamei*.

**Material and methods**

A control diet (Con) was formulated to contain 10% fish meal (anchovy and tuna byproduct) and 4% cod liver oil. The experimental diets were prepared by replacing the fish oil in Con diet with SCH oil and soybean oil at graded levels of 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5% and 100% (designated as S12.5, S25, S37.5, S50, S62.5, S75, S87.5 and S100, respectively). Total 945 shrimp (0.09 ± 0.01 g) were randomly stocked into 27 tanks (240 L) with 35 shrimp per tank. Triplicate groups of shrimp were fed one of the diets four times daily for 56 days. After the feeding trial, each dietary treatment were randomly stocked into 27 tanks (120 L) with 18 shrimp per tank in triplicate groups for a challenge test. *Vibrio parahaemolyticus* suspension was added to each tank to achieve a final bacterial concentration of $3.5\times10^5$ CFU/mL. The challenge test was carried out over a period of 10 days (240 h) monitoring the mortality hourly.

**Results**

Growth performance (final body weight, weight gain and specific growth rate) and feed conversion ratio were significantly improved in all SCH oil groups than in Con group. Lipase activity was significantly higher in shrimp fed S50, S62.5, S75, S87.5 and S100 diets than in those fed Con diet. Fatty acid oxidation activity was significantly higher in shrimp fed S87.5 and S100 diets than in shrimp fed Con and S12.5 diets. Superoxide dismutase activity was significantly higher in shrimp fed S25 to S100 diets than in shrimp fed Con and S12.5 diets. Catalase activity was significantly higher in all SCH-supplemented groups compared to that of Con group. Malondialdehyde levels were significantly lower in shrimp fed S12.5 to S87.5 diets than in those fed Con diet. Total antioxidant capacity was significantly higher in shrimp fed S50, S62.5 and S87.5 diets than in shrimp fed Con diet. Triglyceride and aspartate aminotransferase levels were significantly lower in all SCH-supplemented groups compared to those in Con group. The proportion of *Vibrio* spp. on TCBS agar was significantly lower in shrimp fed S50 to S100 diets than in shrimp fed Con diet. Total hemocyte count was significantly higher in shrimp fed S25 and S37.5 diets compared to those in Con group. The Hyalinocyte count was significantly higher in shrimp fed S25, S37.5, S50 and S75 diets compared to that in Con group. During the *V. parahaemolyticus* challenge test, Con group showed significantly lower survival compared to S12.5 to S87.5 groups.

**Conclusion**

Dietary fish oil replacement with SCH oil and soybean oil had positive effects on growth performance, feed efficiency, digestive enzyme activity, antioxidant capacity, immune responses, gut microbiota balance and disease resistance in *P. vannamei*. Based on quadratic regression analysis of weight gain, the optimum replacement level of fish oil by SCH oil in shrimp diet was estimated to be approximately 70%.

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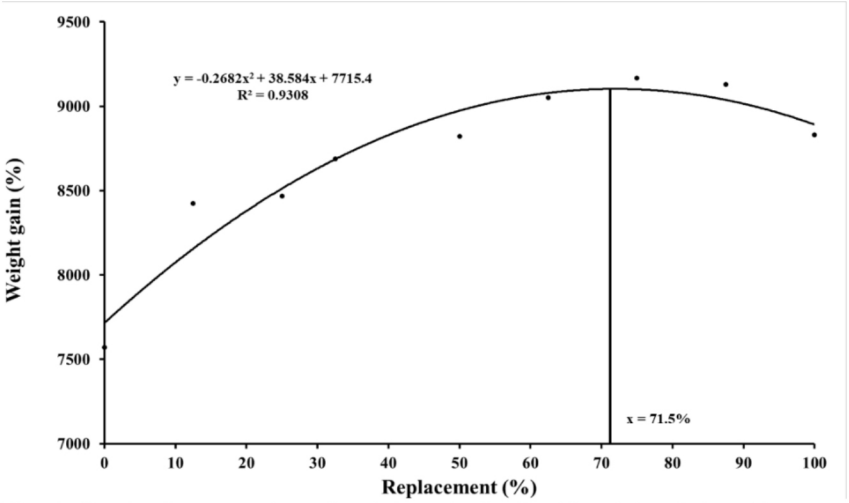


Fig. 1. Quadratic regression of weight gain for Pacific white shrimp *Penaeus vannamei* fed the experimental diets for 8 weeks.

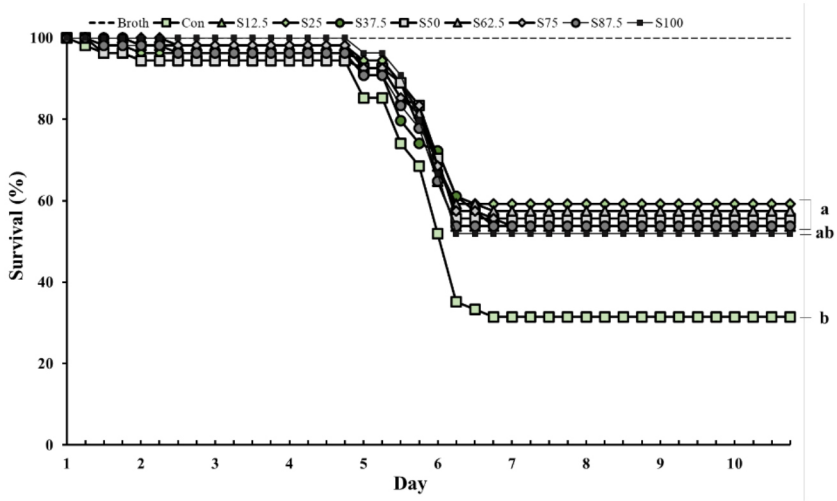


Fig. 2. Cumulative survival of Pacific white shrimp *Penaeus vannamei* during the *Vibrio parahaemolyticus* challenge test. The shrimp were fed the experimental diets for 8 weeks before the challenge.

# A COMPARATIVE STUDY OF UTILIZATION OF DIETARY COENZYME Q10 AND ITS MICELLAR ENCAPSULATION FOR PACIFIC WHITE SHRIMP *Penaeus vannamei*

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## Introduction

Coenzyme Q10 (CoQ10) is a hydrophobic antioxidant that has gained attention as a functional feed additive in aquaculture. Micellar encapsulation is an effective technique to enhance intestinal absorption of hydrophobic compounds such as CoQ10. Despite its potential, very limited information is available regarding the comparative effects of dietary CoQ10 and its micellar form for the shrimp. This study was conducted to evaluate the effects of dietary supplementation of CoQ10 and micellar CoQ10 (MQ10) on growth performance, feed utilization, immune response, antioxidant capacity and resistance to acute hepatopancreatic necrosis disease in the shrimp.

## Material and methods

A control diet (Con) was formulated to contain 15% fish meal (anchovy and tuna byproduct) and 4% cod liver oil. The experimental diets included the Con diet supplemented with CoQ10 at 20 and 40 mg/kg (designated as CQ2 and CQ4, respectively), as well as with MQ10 at levels of 10, 20, 40, 80 and 160 mg/kg (designated as MQ1, MQ2, MQ4, MQ8 and MQ16, respectively). Total 720 shrimp (0.70 ± 0.01 g) were randomly stocked into 24 tanks (240 L) with 30 shrimp per tank. Triplicate groups of the shrimp were fed one of the diets six times daily for 56 days. After the feeding trial, *Vibrio parahaemolyticus* suspension was added to each tank to achieve a final bacterial concentration of $3.0\times10^5$ CFU/mL for a challenge test. The challenge test was carried out for 8 days (192 h) monitoring the mortality hourly.

## Results

Final body weight and specific growth rate were significantly higher in shrimp fed CQ2, MQ2, MQ4, MQ8 and MQ16 diet than in shrimp fed Con diet (Table. 1). Feed conversion ratio, protein efficiency ratio and survival were not significantly affected by dietary administration of CoQ10 and MQ10. Digestive enzyme activities (trypsin, chymotrypsin, and lipase) were significantly increased in MQ8 than in all other groups and Con group. Lysozyme activity was significantly higher in all MQ10 supplemented groups than in Con group. Phagocytosis activity was significantly higher in shrimp fed CQ2, CQ4, MQ8 and MQ16 diets than in shrimp fed Con diet. Antioxidant activities (superoxide dismutase and catalase) were significantly improved in CQ2, CQ4 and all MQ10 groups than in Con group. During the *V. parahaemolyticus* challenge test, Con group showed significantly lower survival compared to CQ4, MQ2, MQ4, MQ8 and MQ16 groups.

## Conclusion

Dietary supplementation of CoQ10 had positive effects on non-specific immunity, antioxidant capacity and disease resistance of *P. vannamei*. Micellar encapsulation enhanced the absorption and utilization of CoQ10 in the shrimp, suggesting that MQ10 is a promising delivery form. These findings indicate that CoQ10 is a potential natural feed additive for the improvement of the shrimp health and disease resistance.

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Table 1. Growth performance, feed utilization and survival of *P. vannamei* fed the experimental diets for 56 days.

| Treatments | [1]FBW (g) | [2]SGR (%/day) | [3]FCR | [4]PER | Survival (%) |
|---|---|---|---|---|---|
| Con | $9.07 \pm 0.06^{c}$ | $4.58 \pm 0.04^{c}$ | $1.81 \pm 0.02$ | $1.54 \pm 0.02$ | $93.33 \pm 3.33$ |
| Q2 | $10.05 \pm 0.27^{a}$ | $4.75 \pm 0.04^{a}$ | $1.76 \pm 0.05$ | $1.55 \pm 0.04$ | $94.44 \pm 5.09$ |
| CQ4 | $9.53 \pm 0.09^{bc}$ | $4.65 \pm 0.04^{bc}$ | $1.79 \pm 0.03$ | $1.51 \pm 0.03$ | $91.11 \pm 1.92$ |
| MQ1 | $9.52 \pm 0.15^{bc}$ | $4.65 \pm 0.03^{bc}$ | $1.76 \pm 0.09$ | $1.55 \pm 0.08$ | $92.22 \pm 1.92$ |
| MQ2 | $9.86 \pm 0.28^{ab}$ | $4.73 \pm 0.04^{ab}$ | $1.71 \pm 0.13$ | $1.59 \pm 0.13$ | $92.22 \pm 1.92$ |
| MQ4 | $9.95 \pm 0.15^{ab}$ | $4.74 \pm 0.02^{a}$ | $1.76 \pm 0.06$ | $1.55 \pm 0.05$ | $91.11 \pm 1.92$ |
| MQ8 | $10.20 \pm 0.17^{a}$ | $4.78 \pm 0.03^{a}$ | $1.73 \pm 0.05$ | $1.58 \pm 0.04$ | $95.56 \pm 5.09$ |
| MQ16 | $10.01 \pm 0.03^{ab}$ | $4.74 \pm 0.01^{a}$ | $1.71 \pm 0.09$ | $1.58 \pm 0.09$ | $92.22 \pm 1.92$ |

[1]FBW, final body weight (g); [2]SGR, specific growth rate (%/day); [3]FCR, feed conversion ratio (feed intake/wet weight gain); [4]PER, protein efficiency ratio (wet weight gain/total protein given).

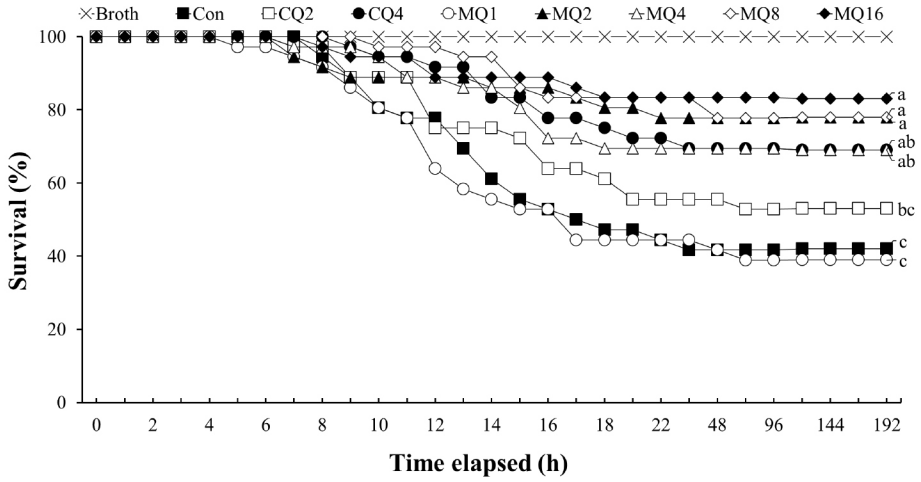


Figure 1. Survival of *P. vannamei* during *V. parahaemolyticus* challenge test after the feeding trial where the shrimp were fed the experimental diets for 56 days.

# METABOLOMICS AND FLOW CYTOMETRY ANALYSES OF HEMOCYTES IN THE PACIFIC OYSTER (*Crassostrea gigas*) UNDER HYPOXIA AND ELEVATED WATER TEMPERATURE CONDITIONS

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## Introduction

The Pacific oyster (*Crassostrea gigas*) is one of the most commercially important aquaculture species worldwide, supporting both global shellfish production and coastal economies. However, its cultivation is increasingly threatened by environmental stressors, particularly those associated with climate change. Hypoxia, often occurring alongside elevated summer water temperatures, can exert severe impacts on aquatic organisms, including mass mortality events in oyster farms.

## Material and Methods

In this study, we examined the metabolomic profiles and cellular responses of hemocytes from *C. gigas* subjected to hypoxia alone or in combination with high seawater temperature stress. Oysters were maintained for 13 days under three conditions: control (normoxia at ambient temperature), hypoxia (1 mg $O_2$/L), and combined stress (28 °C with 1 mg $O_2$/L). Hemocyte metabolite compositions were profiled using gas chromatography–mass spectrometry (GC–MS), and phenotypic characteristics were assessed via flow cytometry.

## Results

Both stress treatments, especially the combined condition, induced marked changes in hemocyte metabolism and functionality. Concentrations of most amino acid metabolites linked to anaerobic respiration and the urea cycle declined, whereas lipid metabolites associated with cell proliferation increased. Flow cytometry revealed a reduction in phagocytic hemocyte populations, which aligned with lower levels of lipid metabolites related to phagocytosis. Furthermore, distinct lipid metabolite shifts were identified that are indicative of impaired cellular immunity.

## Discussion

Collectively, these results demonstrate that hypoxia and elevated seawater temperature act synergistically to suppress metabolic processes through modifications in anaerobic respiration pathways while concurrently diminishing immune capacity in Pacific oyster hemocytes, potentially contributing to the summer mortality phenomenon.

# ANNUAL SEXUAL CYCLE OF FEMALE STONE MOROCCO (*Pseudorasbora parva*) - AN INVASIVE SPECIES FROM NORTHWEST POLAND

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**Introduction**

Stone morocco (*Pseudorasbora parva*) is a small fish belonging to the cyprinid family (Cyprinidae), native to Southeast Asia. It lives in a variety of habitats, being most abundant in small vegetated channels, ponds and small lakes. In higher densities, it can pose a great threat to rare and disappearing fish species, causes a reduction in biodiversity, and spreads parasitic diseases. In Poland since 1990, now very widespread. Stone morocco has contributed to the reduction in abundance and even disappearance of some indigenous fish species of the cyprinid family by eating their eggs and juvenile stages of fish, causes a reduction in biodiversity, as well as the spread of parasitic diseases. The present study aimed to (I) analyse the reproductive cycle of both stone moroko sexes based on microscopic observations; (II) assess the reproductive potential of a population based on a small watercourse in Central Europe. Histological and microscopic analysis of female gonads allows us to assess the maturity of female gonads, determine the spawning period and the rate of gonad recovery.

**Materials and methods**

The study material consisted of 234 females collected from the Wardynka River (Oder basin, northwest Poland). The age of the fish studied, was estimated by scale analysis. Histological analyses of gonad preparations were performed using a standard paraffin technique, gonads were estimated on a 6-grade maturity scale.

**Result**

Standard length and weight of stone morocco females averaged 46.2 ± 12.4 mm and 1.85 ± 1.66 g, respectively. Females were 0+ - 5+ in age. From September to February, gonads with oocytes in previtellogenesis and early vitellogenesis with degenerating oocytes were observed. Multiple portions spawning took place from April to mid-August. Females had the highest GSI in May, averaging 8.55% (0.53-25.68%) and the lowest in January, 1.79% (0.21-2.91%). GSI of spawning females averaged 12.33% (8.23-25.68%). The wide range in the GSI of spawning females is due to the fact that some of the females were after laying a portion of oocytes and were preparing (at different stages) to lay more portions. The average absolute fecundity is 1372 egg per female (range 225-6620) and the relative fecundity is 1691 egg per g (range 268-4543). The smallest spawning female was 2.5 cm (SL).

**Conclusion**

The study population in the settled new habitat showed a higher reproductive potential than native populations of stone morocco. The fish were characterized by very rapid sexual maturity as early as in the first year of life, high fecundity, abortive spawning, and a long-lasting four-month reproductive period, which allows for a rapid increase in population size and density in the new area. Reproductive potential, together with low subsistence requirements and adaptability to different habitat conditions, contribute to the colonization success of this species in a new habitat.

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# UV-B LIGHT STIMULATES THE PRODUCTION OF VITAMIN $D_3$ IN ATLANTIC SALMON

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## Introduction

Vitamin D is an essential fat-soluble micronutrient in animals, and the two most important derivates are vitamin $D_2$ (ergocalciferol) and vitamin $D_3$ (cholecalciferol). The $D_2$ originates from vegetable and fungal origins and the D3 from animal origins (Cardwell et al., 2018). Mammals can additionally synthesize pre-vitamin D3 in the skin from 7-dehydrocholesterol (7-DHC). This is done in humans by UV-B radiation of 290-315 nm which causes the epidermal 7-DHC to rearrange and form pre-vitamin $D_3$. (Holick et al., 1977). This is also common among land vertebrates such as lizards and birds and also among some insects (Oonincx et al., 2018).

The main origin of D3 in fish such as Atlantic salmon, has been thought to be via the food chain (Lock et al., 2009). It has been stated that fish is fully dependent on dietary D3 (Lock et al., 2009; Soto-Dávila et al., 2020).

We wanted to test whether UV-B light stimulated the production of vitamin D3 in Atlantic salmon.

## Material and methods

For the trials, 4 indoor holding tanks (3 x 3 m, 8 angular) with a water volume of ~ 10.8 m3 (depth 1.2 m) were used. The indoor area was enlightened with ordinary white light from tube lights in the roof, at a continuous 24/7 light regime for all tanks. In addition, two UV-B-light sources (LumenIZE Pro T5 Forest 6% 54W, Arcadia reptile, Great Britain) were installed 30 cm above the water surface in two of the tanks (UV-B exposed fish) at a 24/7 light regime. For comparison fish from two random tanks were used (control/non-exposed fish). Atlantic salmon were reared from roe (Benchmark genetics, Norway) at Salmon Evolutions smolt facility at Dale in Norway following the normal smolt production. The average weight in start of the trial was 6g. Two separate trials were done.

Fish were sampled from the 4 indoor tanks at 3 separate occasions in both trials, at day 0 (start of the trial), day 31, and day 77 in trial 1 and day 0, day 27, and day 68 in trial 2. Fish were tawed, and filets with pin bones extracted was analysed for Cholecalciferol ($D_3$) by Eurofins Food & Feed Testing Norway AS (EN 12821:2009 mod.). The method consists of saponification and extraction followed by HPLC analysis and detection.

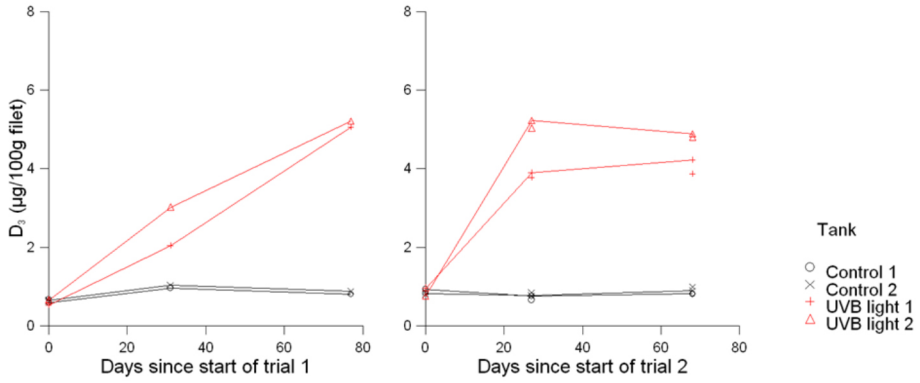


Figure: $D_3$ levels in samples from the tanks plotted against day since the trial started.

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**Results**

The level of D3 in muscle was markedly affected by the presence of a UV-B light source. Filet $D_3$ levels started out at ≤ 1 µg/100 g in all tanks during both trials. $D_3$ levels in fish exposed to UV-B increased during both trials, whereas in the control groups $D_3$ levels remained at low levels throughout. Fish from UV-B treated tanks had significantly higher $D_3$ levels than fish from the control tanks.

**Conclusion**

The groups exposed to UV-B light had 2,5 to 6 times higher Vitamin $D_3$ in the muscle after 4 weeks and more than 5 times as much after about 10 weeks than the control groups. The study suggests that Atlantic salmon in the freshwater phase synthesize pre-vitamin $D_3$ from 7-dehydrocholesterol using UV-B light.

In today's salmon farming, where more of the farming occurs in closed and submerged facilities with no or limited access to UV-B from natural sunlight, this knowledge is important.

**Acknowledgements**

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# FROM AQUACULTURE SLUDGE TO MICROALGAE – A VALUE CHAIN ASSESSMENT

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## Introduction

With the rapid growth of the Norwegian aquaculture industry, also the demand for suitable aquaculture waste management is rising. Current waste treatment practices include both partial release into the surrounding water as well as capture, dewatering and shipment to other countries such as Denmark or Vietnam where it is used in biogas production or as fertilizer. Here we evaluated a waste-treatment process in Norway, where anaerobic digestion of the fish sludge was used for biogas production in Norway and the leftover nutrients, such as nitrogen (N) and phosphorous (P) from the aquaculture effluent water were used for microalgae cultivation. The final products from this proposed value chain are energy and heat from biogas, liquid digestate (organic fertilizer), as well as microalgae biomass. To create a better understanding of the environmental and economic benefits of the proposed value chain, as well as any drawbacks, a life cycle analysis as well as preliminary economic assessment was performed.

## Material and Methods

Four different scenarios were investigated: 1) representing the current situation of sludge treatment from an average fish production site with sludge-capturing mechanisms and shipping 50% of the captured sludge to Denmark for biogas production, 2) assuming 100% of the captured sludge to be shipped to Denmark for biogas production, 3) nutrients of the reject water from sludge dewatering are remediated through microalgae cultivation, and the dewatered fish sludge is used as sole substrate for biogas production in Norway, and 4) the same as in 3, though with the distinction that the dewatered fish sludge is added as an additional feedstock to a municipal anaerobic digestion plant in Norway. All scenarios were based on the aquaculture waste streams from an assumed average-sized fish production facility located on the west coast of Norway. A life cycle assessment was performed to compare the potential environmental impact of these four different fish sludge treatment scenarios with each other and identify environmental hotspots in the proposed value chains. The assessment followed the ISO14040 and ISO 14044 guidelines. One year of treatment of fish sludge and reject water from an average salmon production site using Recirculating Aquaculture System (RAS) and producing 5000 tons fish per year (Bregnballe, 2022), was chosen as the functional unit for the LCA. Moreover, A preliminary economic assessment was performed to quantify the differences in operational costs of the scenarios.

## Results and Discussion

Shipping and treatment of 100% of the fish sludge in Denmark, as described in scenario 2, gave lower environmental impacts than the baseline scenario where only 50% of the fish sludge was shipped and treated in Denmark. Geographical differences in electricity sources in Norway and Denmark are a major factor that determine the environmental sustainability. System expansion, for example to account for the lack of imported sludge in Denmark and subsequent local energy production (biogas) compensated by energy from the local grid, was a major contributor to the environmental impacts. Even though the amount of energy produced from the biogas production in scenario 2 and 4 was the same, the compensated energy coming from the local grid had a significantly different environmental footprint, with the Danish energy mix based on wind power (58%), biofuels (16%), 10% solar power, and 16% non-renewable energy, and the Norwegian energy mix based for 89% on hydropower, and another 10% based on other low-carbon energy sources.

In scenarios 1 and 2, the system was expanded with the production of microalgae on commercial fertilizer and electricity from the national Norwegian grid alone. This was a major contributor to the environmental impacts and had a greater impact than the treatment of fish sludge in Denmark. When all scenarios were compared without a system expansion for compensation of local energy production in either Norway or Denmark, scenarios 3 and 4 became the most environmentally sustainable scenarios in all impact categories.

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Scenario 4, where fish sludge was co-digested with other feedstock, showed a potentially lower environmental impact than when fish sludge was the sole substrate for anaerobic digestion (scenario 3), due to variations in the biogas productivity, and thus production of less energy that could be used in the microalgae production. Optimisation of anaerobic digestion with high inclusion levels of fish sludge can play an important role here.

As expected, in the scenarios where microalgae were cultivated on aquaculture wastewater and thus recovered the nitrogen from this water, the impact category marine eutrophication was clearly improved.

The direct comparison of only the treatment of fish sludge for biogas production (when using co-digestion with other feedstocks) showed that local treatment of the sludge in Norway resulted in less environmental impact in most of the impact categories compared to treatment in Denmark. This is mainly caused by a lower energy consumption (as no drying of fish sludge is necessary, only dewatering up to 20 % DW) and shorter transport distances (no shipment, only transport by road). However, infrastructure for anaerobic digestion is far less developed in Norway, and the use of the produced digestate needs also to be taken into account.

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# REVIEW OF GROWTH MODELS FOR POST-SMOLT TO ADULT ATLANTIC SALMON *Salmo salar*

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## Introduction

Atlantic salmon (*Salmo salar*) is among the top ten most produced and valuable marine fish worldwide in the aquaculture industry, with Norway producing 1.5 million metric tonnes (MMT) in 2023, making it the leading global producer. However, this high level of production has been accompanied by significant challenges, notably the issue of salmon louse (*Lepeophtheirus salmonis*), a parasite responsible for significant welfare concerns and an estimated economic impact of around €610 million annually in Norway. The problem has seemingly escalated due to increased production volumes, reduced efficiency of treatments and rising sea surface temperatures (SST). For instance, in Northern Norway, record-high SSTs during July and August 2024 led to a surge in salmon lice larvae production. Rising SST can also lead to a higher frequency of harmful algal blooms and jellyfish blooms. SST are projected to increase by 1-3 °C this century and therefore understanding the potential future scale of salmon lice infestations, especially under conditions of higher SSTs anticipated with climate change, is crucial.

One effective approach to assess the impact of rising salmon lice populations linked to increased SSTs is through simulation studies. Simulation-based approaches, particularly growth models, provide valuable tools for linking environmental changes to fish growth, mortality, and economic outcomes. While empirical models like specific growth rate (SGR) and thermal growth coefficient (TGC) offer simplicity for operational use, mechanistic bioenergetic models better capture complex interactions between temperature, feeding, metabolism, and parasite burdens. Integrated frameworks that combine bioenergetics with epidemiological and economic modules are especially suited for evaluating adaptation strategies, as they can simultaneously simulate reduced growth, lice-induced mortality, and treatment-related losses to inform robust, climate-resilient management of Norwegian salmon aquaculture. To support climate change adaptation (CCA) in Norwegian Atlantic salmon aquaculture, our review sought growth-modelling frameworks that can simulate the combined impacts of rising SST and heightened salmon-lice infestation while also incorporating farm-management levers, growth, mortality and cost from the post-smolt to adult phase.

## Method

In this review, we compiled and synthesised literature on growth models for post-smolt to adult salmonids, following PRISMA guidelines. An initial Web of Science and Oria search targeting “Atlantic salmon” and “growth model*” returned only 12 relevant papers, so the scope was broadened to include other salmonids (salmon, trout, charr) to capture a wider range of modelling approaches applicable to Atlantic salmon, while excluding non-salmonid species from multi-species studies. The expanded search retrieved 267 papers, of which 83 met inclusion criteria after screening titles, abstracts, and methods. These studies were classified by species, production system, and whether their models measured growth, weight, mortality, and cost, and incorporated temperature or salmon lice as drivers, with growth and temperature as the minimum requirements for recommendation. From this set, 28 key studies most relevant to the review’s aims were examined in depth, grouped into main model categories, and analysed to identify the most suitable frameworks for simulating climate- and parasite-related impacts on Atlantic salmon.

## Results

The literature on post-smolt to adult Atlantic salmon growth models spans a spectrum from simple empirical frameworks to fully integrated farm-scale systems, differing in their ability to capture environmental and biological stressors. Empirical index models, such as TGC, provide rapid temperature-based growth estimates but generally ignore parasite effects. Phenomenological or allometric curve models fit observed growth trajectories, while bioenergetic mass-balance models explicitly link feeding, metabolism, temperature, and parasite loads to produce more mechanistic projections. Nutrient-budget/DEB-style models extend these capabilities by tracking protein, lipid, and other nutrient fluxes, enabling precision feeding and environmental

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impact assessments. At the most comprehensive level, integrated farm- or system-scale platforms embed bioenergetics within epidemiological and economic modules, allowing simulation of lice impacts, mortality, and production costs under varying SST scenarios. Spatially explicit and tissue turnover–based approaches add further refinement by incorporating habitat-specific parameters and reproductive energy costs. Among the 83 studies screened, most measured growth (often converted to weight), but relatively few addressed mortality, costs, or health effects, and only one explicitly linked lice to growth, highlighting a need for more holistic models. The 28 most relevant studies were grouped into five categories: (1) empirical/index models, (2) phenomenological/allometric curve models, (3) bioenergetic (mass-balance) models, (4) nutrient-budget/DEB-style models, and (5) integrated farm- or system-scale models.

**Discussion**

Choosing an optimal model for predicting Atlantic salmon performance under climate change requires balancing simplicity, mechanistic accuracy, and the ability to incorporate multiple stressors. Empirical indices like TGC are effective for rapid forecasts but cannot account for interactions between temperature, feeding, and parasite loads. Bioenergetic models, particularly when integrated with epidemiological and economic modules, allow scenario testing for feed strategies, lice-induced growth suppression, and treatment-related mortality, while incorporating spatial, temporal, and physiological dynamics. Stage-structured epidemiological models are good for simulating lice pressure, as they capture life-cycle stages, temperature-dependent development, and treatment effects, and can be parameterised for local conditions and climate projections. The most robust framework for sea-cage aquaculture is an integrated farm-scale system embedding a mechanistic bioenergetic core within parasite and economic sub-models, generating simultaneous outputs for growth, mortality, lice burdens, and costs. Such hybrids, already used to optimise treatment timing and assess climate-driven site changes, provide the precision and management flexibility needed for evidence-based decision support in modern salmon farming.

**Conclusion**

This review synthesises empirical, mechanistic, and integrated modelling approaches for post-smolt to adult Atlantic salmon under thermal and parasitic stress. Empirical models are operationally simple but lack mechanistic realism, while bioenergetic models offer greater predictive accuracy at the cost of higher data demands. Integrated bioenergetics–epidemiology–economics frameworks emerge as the most robust for climate adaptation planning, enabling simultaneous assessment of growth suppression, mortality, and economic impacts. Future developments should incorporate tissue turnover and reproductive energetics, integrate spatial–temporal environmental variability to capture site-specific differences, and link biological and economic modules to assess cost-effectiveness of adaptation measures. As warming seas and parasite pressures intensify, adopting holistic, mechanistically grounded models will be essential to maintain productivity, welfare, and profitability in Norwegian salmon aquaculture.

# USE OF β-GLUCANS IN FISH: AN UPDATE ON KEY FINDINGS AND EMERGING APPLICATIONS

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**Introduction**

Aquaculture is rapidly emerging as a vital sector in global food production. However, one of its persistent challenges is effective health management. Increasing fish stocking densities often lead to heightened stress levels, which in turn contribute to the outbreak of serious diseases. While antibiotics have traditionally been used to address these issues, their application is now heavily regulated or even banned in many regions. As a result, there is a clear need for alternative strategies to enhance immune responses in fish. One promising approach involves the use of β-glucans, which have been studied for their immunostimulatory effects in fish for several decades.

This presentation will highlight key findings on the use of β-glucans in fish, demonstrating increased survival rates across various species when challenged with a broad range of bacterial pathogens. Data will be presented for both cold-water and tropical fish species. We will also discuss recent advances regarding the role of β-glucans in wound healing - an important factor in disease proliferation - as well as their capacity to enhance vaccine efficacy in salmon. Notably, pre-vaccination intake of β-glucans has shown to improve immune responses to both bacterial and viral infections.

Additionally, we will present findings on the dose-response relationship of β-glucans in tilapia at both optimal and suboptimal water temperatures. These results suggest that water temperature influences how different dosages modulate the immune system. Lastly, we will explore how β-glucans can significantly alter lipid metabolism in fish. Altogether, this presentation aims to provide a comprehensive overview of the multifaceted benefits of incorporating this important additive into fish nutrition, positioning them as a natural, sustainable strategy for improving aquaculture health and performance.

Glucan is a general term used to describe a group of polysaccharide polymers that are classified based on the type of glycosidic linkage they contain—either β- or α-linked. β-glucans derived from different biological sources often exhibit variations in their primary structures and conformations. The primary structure is defined by the type of glycosidic bonds, degree of polymerization, and branching patterns. In contrast, β-glucans conformations can appear as random coils, single helices, or triple helices, depending on factors such as the primary structure, intermolecular interactions, temperature, and solvent conditions.

β-glucans are found across a wide range of organisms, including bacteria, algae, fungi, and plants, each source producing structurally distinct types. Typically, their structure consists of a backbone made up of β-(1,3)- and/or β-(1,4)-linked D-glucopyranosyl units arranged in a non-repeating but non-random manner, with variable side chains branching off the main chain. Because of this structural diversity, the immunomodulatory activity and effectiveness of different β-glucans molecules can vary significantly, even among those with similar molecular weights, structures, and conformations.

Numerous studies have explored correlations between β-glucans biological activity and their molecular structure, size, branching frequency, degree of chemical modification, conformation, and solubility. However, due to often inconsistent or contradictory findings, broad generalizations should be approached with caution. A wide range of concentrations and administration routes, including oral delivery, have been tested, yet no consensus has been reached regarding the optimal source, molecular size, or physicochemical characteristics of β-glucans for immunomodulatory purposes.

**Method**

This presentation will summarize nine recently published scientific articles from leading international journals. These studies were carefully selected to highlight the diverse approaches and applications of β-glucans in fish nutrition. The articles written by Roberti Filho et al 2019, Do-Huu et al 2016, Koch et al 2021 and Fronte et al 2019 will be highlighted in the presentation.

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**Results**

The use of beta-glucan for fish:

1. Improves growth, survival rate against several bacterial pathogens;
2. Long periods of use of β-glucans, at the correct dosage, are beneficial to fish;
3. Acts in the modulation of the immune system and wound healing;
4. Increases tolerance to temperature stress;
5. Acts as a vaccine adjuvant;
6. Is associated with a modulation of the physiology, immunity and microbiota of the fish larvae;
7. Strengthen the salmon lice preventing effect;
8. Presents a synergistic association with vitamins important to antioxidant system;
9. Increases tolerance to stress due to exposure to ammonia;

**Conclusion**

To date, more than 3,000 studies have investigated the effects of beta-glucans on immune responses in fish. Despite this extensive body of research, several key questions remain unresolved. In particular, there is still limited understanding of the receptors involved in beta-glucan recognition and the downstream signaling pathways in teleosts. As a result, it remains unclear whether the observed immunostimulatory effects are due to direct action on leukocytes or indirect mechanisms—such as alterations in the gut microbiota composition.

Most existing studies have focused on short-term immune responses, typically lasting from a few days to several weeks. However, emerging research in innate immunity suggests that beta-glucans may also exert longer-lasting effects, potentially through a mechanism known as “trained immunity,” which may provide prolonged enhancement of immune function.

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# EFFECTS OF DIETARY SOLUBILIZED MANNANOLIGOSACCHARIDES ON PERFORMANCE, HEALTH STATUS, NITROGEN STRESS RESISTANCE, AND ECONOMIC BENEFITS IN PACIFIC WHITE SHRIMP, *Penaeus vannamei*, RAISED IN AN INTENSIVE SYNBIOTIC SYSTEM

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**Introduction**

As shrimp farming techniques have evolved and intensified, concerns have arisen regarding the physiological stress and immune status of farmed shrimp. Historically, chemotherapy, such as the use of antibiotics, has been used to control disease outbreaks in shrimp farming. This has resulted in the broad spread of antibiotic-resistant pathogens and significant environmental contamination. As an alternative, a new generation of MOS-based prebiotics is emerging that achieves solubilization of the mannan layer within the yeast cell wall, making it more accessible and partially exposing the glucan layer. This synergistic process enhances the overall efficacy of these prebiotics. β-Glucan is a complex polysaccharide that has been shown to have remarkable immunostimulatory, anti-inflammatory and antioxidant properties in aquatic animals. The objective of this study is to evaluate the efficacy of second-generation MOS (solubilized MOS) at different levels in the diet of *P. vannamei*.

**Material and methods**

A control diet (C) containing commercial ingredients from the Brazilian feed industry was formulated to meet the recommended nutritional requirements for juvenile Penaeus vannamei. The diet was designed to be isoproteinic (360 g kg-1), and isoenergetic (4400 kcal kg-1). The commercial mannanoligosaccharides (MOS) source consists of 27.2% solubilized mannanoligosaccharides (Hypergen, Biorigin, São Paulo, Brazil). Additionally, three experimental diets were formulated, based on the control diet, incorporating a commercial MOS source to replace wheat flour at inclusion levels of 1.0g (0.27g mannans), 2.0 (0.54g mannans), and 4.0 g (1.08g mannans), of commercial solubilized MOS per kg feed-1. These diets were designated as M1.0, M2.0 and M4.0, respectively. The study was conducted over 60 days at the Laboratório de Carcinicultura (LACAR), of the Departamento de Pesca e Aquicultura (DEPAq) at the Universidade Federal Rural de Pernambuco (UFRPE), Brazil. During the feeding trial, the shrimps (initial weight 3.0 ± 0.04 g) were fed three times daily (8.00 am, 01:00 pm and 5:00 pm) with the experimental diets.

**Results**

At the end of the trial, higher performance (table 1) was observed in shrimp fed the diet supplemented with 1.0g solubilized MOS kg-1 ($p < 0.05$). MOS (1.0 g kg-1, 2.0 g kg-1) supplementation resulted in a significant improvement in gut morphology as evidenced by increased villus and enterocyte heights. In the ammonia (table 2) and nitrite stress tests, shrimp fed the MOS supplement showed improved resistance as indicated by higher survival rates and lower histological alterations in the gills (table 3) during stress periods. The economic benefits in the M1.0 and M2.0 treatment were the most favorable. The results show that MOS supplementation improves shrimp performance, shrimp gut health, and economic benefits.

**Conclusion**

To optimize economic and health benefits to *P. vannamei* in intensive synbiotic systems, supplementation with solubilized MOS at a level of 1.0 to 2.0 $kg^{-1}$ feed is recommended.

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Table 1. Performance of *P. vannamei* juveniles fed control and test diets with commercially solubilized mannanoligosaccharides in a synbiotic system for 60 days.

| Variables | Treatments | | | |
|---|---|---|---|---|
| | C | M1.0 | M2.0 | M4.0 |
| Final weight (g) | 8.86 ± 0.13$^{c}$ | 10.87 ± 0.26$^{a}$ | 9.30 ± 0.13$^{b}$ | 8.39 ± 0.17$^{d}$ |
| Survival (%) | 90.50 ± 1.50$^{a}$ | 91.67 ± 5.51$^{a}$ | 93.00 ± 3.00$^{a}$ | 73.00 ± 2.00$^{b}$ |
| Growth week g$^{-1}$ | 0.68 ± 0.01$^{b}$ | 0.91 ± 0.03$^{a}$ | 0.73 ± 0.02$^{b}$ | 0.63 ± 0.02$^{c}$ |
| kg m$^{-3}$ | 1.0 ± 0.01$^{c}$ | 1.25 ± 0.09$^{a}$ | 1.08 ± 0.02$^{b}$ | 0.77 ± 0.02$^{d}$ |
| kg ha$^{-1}$ | 8,014 ± 131$^{c}$ | 9,967 ± 719$^{a}$ | 8,644 ± 161$^{b}$ | 6,182 ± 51$^{d}$ |
| FCR | 1.70 ± 0.01$^{b}$ | 1.20 ± 0.14$^{d}$ | 1.50 ± 0.02$^{c}$ | 2.10 ± 0.10$^{a}$ |

Table 2. Shrimp *P. vannamei* survival (%) after ammonia nitrogen stress tests feeding with dietary commercially solubilized mannanoligosaccharides.

| Exposure time | Treatments | | | |
|---|---|---|---|---|
| | C | M1.0 | M2.0 | M4.0 |
| 24hrs | 100 ± 0.0$^{a}$ | 94.4 ± 3.9$^{a}$ | 97.2 ± 3.9$^{a}$ | 91.7 ± 6.8$^{a}$ |
| 48hrs | 80.6 ± 10.4$^{a}$ | 91.7 ± 6.8$^{a}$ | 88.9 ± 3.9$^{a}$ | 83.3 ± 6.8$^{a}$ |
| 72hrs | 61.1 ± 7.9$^{b}$ | 83.3 ± 0.0$^{a}$ | 83.3 ± 0.0$^{a}$ | 75.0 ± 6.8$^{ab}$ |
| 96hrs | 47.2 ± 7.9$^{b}$ | 80.6 ± 3.9$^{a}$ | 69.4 ± 7.9$^{a}$ | 55.5 ± 3.9$^{b}$ |

Table 3. Histological alteration index values of gills of *P. vannamei* challenged with N-NH3 and N-NO2 stress tests.

| Stage | | Treatments | | | |
|---|---|---|---|---|---|
| | | C | M1.0 | M2.0 | M4.0 |
| Stage I | Normal tissue function | 30.8 % | 48.3 % | 32.4 % | 31.4 % |
| Stage II | Mild to moderate alteration | 7.7% | 6.9 % | 8.1 % | 5.7 % |
| | Moderate to severe alteration | 46.2% | 44.8% | 56.8% | 42.9% |
| Stage III | Severe alteration | 15.38 % | 0.0 % | 2.7 % | 20.0 % |
| | Irreparable alteration | 0.0% | 0.0% | 0.0% | 0.0% |
| | HAI | 111.3 ± 6.7$^{a}$ | 14.7±5.1$^{b}$ | 25.5±7.6$^{b}$ | 107.8±8. |

# EVALUATION OF THE INFLUENCE OF SELECTED FACTORS ON THE QUALITY OF GAMETES AND EMBRYONIC DEVELOPMENT OF GRAYLING (*Thymallus thymallus*)

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## Introduction

Populations of grayling are declining across Europe due to a number of factors including climate change, pollution, regulation of watercourses and predation by piscivores (Persat, 1996; Uiblein in sod., 2005; Turek in sod., 2013; Didenko in sod., 2018). One measure to mitigate this decline is artificial breeding in fish farms and reintroduction into watercourses (Nykänen in sod., 2001; Ovidio in sod., 2004; Szmyt in sod., 2013). As grayling is not a commercially important species, breeding protocols for farms have not yet been optimized and the reproductive parameters of this species are poorly known, resulting in lower breeding success compared to more commercially interesting species. In our study, we therefore investigated the influence of various factors, with a focus on feed, on the quality of grayling gametes in order to improve the breeding process in fish farms.

## Materials and methods

The practical part of the study was carried out in two consecutive years at the Soča fish farm in Kobarid. At the beginning of the experiment, the breeding stock of grayling was divided into four tanks. In the first year, 50 fish aged three years (fish between the first and second spawning - first-year fish) were placed in each tank. In the second year, we continued the study with one-year-old fish that had survived the first year. Since there is no optimized feed specifically developed for grayling, we tested three different commercial feeds in the experiment. These included:: the lower-carbohydrate commercial salmonid feed INICIO 702 (feed 1), the carbohydrate-rich commercial feed INICIO Plus (feed 2) and a carotenoid-enriched feed, EFICIO Enviro 978 from the same manufacturer (feed 3). The main parameters used to assess the quality of the gametes were the fertilisation rate of the eggs after two hours, the survival rate to the eye stage and the hatching rate of the embryos. Egg quality was further assessed by analysing their biochemical composition, while sperm quality was determined by measuring progressive motility, sperm velocity and sperm concentration in seminal fluid.

## Results

When analysing the results, we found that the average fertilization rate of grayling was 70%, the survival rate to the embryonic stage with developed eyes was 25%, while the hatching rate of larvae was only 5%. Most eggs were lost by the end of gastrulation. We found that several factors influence the rearing of grayling on the farm, one of which is the age of the broodstock. The first time spawners had almost half the average absolute fecundity of the older fish, which was also reflected in the characteristics of their eggs. The eggs of the first fish were smaller (2.5 mm) and lighter (25 mg) than those of the older fish, whose eggs measured 2.7 mm and weighed 32 mg. The greatest differences between the eggs of first time spawners and older fish were found in the group of polyunsaturated fatty acids (PUFA) such as EPA, C18:4 n-3, C18:3 n-6 and C20:4 n-3, the content of which was lower in the eggs of first time spawners. The higher DHA/EPA ratio in the eggs of first time spawners makes them more likely that they will develop early mortality syndrome. The influence of age was also evident in males. Males at three years of age had significantly lower sperm velocity and progressive motility than males four years of age. In contrast, male grayling in the first year of the study had a higher sperm concentration in the seminal fluid than in the second year.

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The effect of feed on the reproduction of grayling was less than expected. The survival rate of embryos and larvae showed no differences depending on the type of feed fed to the broodstock, and there were also no differences in the average absolute fecundity. Nevertheless, we observed certain effects of the feed on the fatty acid composition of the eggs and the sperm concentration in the seminal fluid. In the females fed the carbohydrate-rich diet (feed 2), we observed lower levels of n-6 PUFA and higher levels of long-chain PUFA (LC PUFA) and long-chain n-3 PUFA (LC n-3 PUFA) in the biochemical composition of the eggs than in the other two groups. However, a higher sperm concentration in the seminal fluid was found in males fed a higher proportion of vegetable fats than in males fed a higher proportion of animal fats. A number of developmental deformities were observed In farm-raised eggs we noticed a number of developmental abnormalities such as the development of the head, eyes, jaws, malformed and curved spine and a deformed tail. These malformations did not occur in the wild-origin roe.

## Conclusion

We conclude that none of the commercial feeds used significantly improved the reproductive parameters of grayling. This may be attributed to the fact that the feeds were not specifically formulated to meet the nutritional requirements of this species. For successful grayling rearing in fish farms, new diets should be developed based on the biochemical composition of grayling eggs.

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# DIETARY REPLACEMENT OF FISH OIL WITH DHA-RICH ALGAE LIQUID SUSPENSION: IMPACT ON GROWTH, FILLET SENSORY PROFILE, AND IMMUNE RESPONSE IN ATLANTIC SALMON

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***Introduction:***
While fishmeal (FM) and fish oil (FO) offer optimal nutritional profiles for most farmed fish, their reliance on wild-caught stocks presents significant environmental challenges. In response, over the past decades, the aquaculture sector systematically re-evaluates feed formulations using potentially more sustainable alternatives, to reduce its dependency on capture fisheries. The shift to alternative ingredients affects key components like ω-3 long chain polyunsaturated fatty acids (LC-PUFAs), crucial for fish health and the nutritional value of the final product. Among them, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to modulate immune cell activity—including macrophages, neutrophils, T-cells, and others—by enhancing phagocytosis, cytokine and chemokine secretion, and cellular polarization. Recent studies have shown that the regulation of fatty acid metabolism-related genes (Xue *et al.*, 2020), as well as immune and disease resistance-related genes (Santigosa *et al.*, 2023), is affected when fish are fed diets with altered EPA:DHA ratios. These findings underline the critical role of EPA and DHA not only in growth and survival, but also in immune competence and overall fish welfare.

Within the Millennial Salmon project (Research Council of Norway #319987), we investigated the potential of novel feed ingredients to replace FM and FO in Atlantic salmon diets without compromising fish performance or health. The Millennial salmon project is a strategic partnership among world-leading European organisations spanning the value chain of the salmon farming industry. A key focus of the project was the evaluation of AlgaPrime™ DHA LS, a DHA-rich microalgae oil suspension (from *Schizochytrium sp*.) produced by Corbion, as an alternative to FO in salmon diets.

***Material & Methods:***
Atlantic salmon post-smolts (600 g) were reared to commercial size (~3.5 kg) in 20 sea cages in Mowi's field trial station (Averøy, Norway) using 6 diets where EPA+DHA from FO was replaced 0%, 20%, 40%, 60%, 80% and to 100%, with DHA from AlgaPrime™ DHA Liquid Suspension (DHA-Algae). Fish growth, health, welfare and different tissue proximate composition were assessed by Nofima, while smoked fillet quality was evaluated by both Nofima's professional sensory panel and Labeyrie Fine Foods laboratories. To investigate potential immunological effects of varying EPA:DHA ratios in the diet, head kidney macrophages were isolated from fish in the cage trial and stimulated *in vitro* with inactivated *Moritella viscosa* to mimic a bacterial challenge. Two timepoints (12 and 48 hours) were selected, representing the early and the late phase of inflammation, to assess whether elevated dietary DHA/EPA modulates the gene expression of immune and inflammatory response markers.

***Results & Discussion:***
Results demonstrated that 100% dietary FO replacement with DHA-Algae had no negative effect on salmon's performance parameters. Whole-body ω-3 content increased in proportion to dietary DHA-Algae inclusion, whereas the sum of fillet EPA+DHA levels gradually increased with replacement of FO with DHA-Algae in the diet, statistically significantly only between the control and 40% FO/ 60% DHA-Algae. These findings align with a similar study by Kousoulaki *et al.* (2020), reporting no adverse effects on salmon performance reared in sea cages alongside enhanced DHA content in the flesh replacing FO with *Schizochytrium sp.* biomass. Sensory profiling of both raw and smoked fillets revealed that diet has a subtle influence on flavour attributes (fish, feed, rancid, total flavour intensity) and juiciness. Collectively, these results indicate that DHA-Algae is a nutritionally valuable and organoleptically acceptable alternative to FO in salmon aquafeeds.

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Gene expression profiling of immune-related markers in head kidney macrophages revealed that dietary EPA/DHA levels modulated transcriptional responses following *in vitro* bacterial stimulation. Several genes showed significant or trending regulation by diet, immune stimulation phase (acute or recovery), or both. Notably, *cfdl, lox3, LOC106609000, LOC106594896, LOC101448046, and LOC106584449*—associated with macrophage activation, complement-mediated defence, and inflammatory signalling—exhibited clear time- and diet-dependent responses, supporting roles in innate pathogen sensing and inflammation. This aligns with previous studies showing PUFA-mediated modulation of pathogen recognition receptor (PRR) and cytokine signalling (Li *et al.*, 2020). Diet significantly influenced *cfdl, LOC106609000*, and *LOC101448046*, with *cfdl* and *LOC101448046* showing diet-related differences at both time points. In addition, *LOC106594896* and *LOC106584449* were strongly influenced by both diet and stimulation phase, suggesting roles in cytokine signalling or macrophage metabolic reprogramming—consistent with immune–metabolic shifts described in fish macrophages (Wentzel *et al.,* 2020). During the recovery phase, most responsive genes showed downregulation across diets, with *LOC106594896* in some diets (control and 60%FO) shifting towards increased expression—indicating potential immune resolution and reprogramming. Such patterns support previous findings that dietary DHA/EPA ratios can modulate immune gene expression (Zuo *et al.,* 2012). Together, these shifts reflect the differential regulation of salmon macrophage responses by dietary fatty acids across immune activation and recovery phases.

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MILLENNIAL SALMON

# EXPLORING DIET × STRAIN INTERACTIONS ON THE GUT MICROBIAL STRUCTURE OF EUROPEAN SEABASS: A LONG-READ SEQUENCING APPROACH

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## Introduction

Feed is considered as one of the major factors for sustainable aquaculture development in the world, and its composition directly influences feed intake and efficiency of fed aquatic animal production. At the same time, the dietary composition of the aquafeeds can have a direct impact on the gut microbiota modulation, ultimately affecting host growth and health (Butt & Volkoff, 2019). Besides the diet, various factors, such as host genetics, age and environmental conditions, are also known to have an influence on the gut microbiota of aquatic species (Mougin & Joyce, 2023). Especially the role of genetics on the microbiota composition of aquaculture fish has been less explored, but few studies have shown that host genotype variability can also alter the microbiota composition (Kokou et al., 2018; Piazzon et al., 2020). At the same time, the host genotype or strain can affect the nutritional utilization of the diet and fish growth rates (Li et al., 2019). Whether differences in the feed utilization between different fish strains link to differences in the gut microbiota composition, remains largely unexplored. In the present study, we explored this research question, by testing three strains of European seabass, fed with two diets, differing in macronutrient composition. Moreover, we utilized a long-read sequencing approach targeting the full-length 16S ribosomal gene for the first time in European seabass, in order to evaluate its applicability in characterizing its mucosal microbial communities under different experimental conditions.

## Materials & Methods

A 10-week feeding trial was performed, evaluating two diets with different macronutrient composition: a diet with high carbohydrate content (HOD), and a diet with high fat content (LOD). The diets were formulated to have a similar dietary protein/dietary energy ratio (DP/DE; ~19 kJ/g dry matter). Three strains of European seabass (average weight 67.8g ± 0.58), originating from IFREMER (Palavas-les-Flots, France) were stocked in separate tanks; an Atlantic strain (ATL), an East Mediterranean strain (MEDEAST) and a West Mediterranean strain (MEDW). The fish were fed twice daily (09.00 and 15.00) up to satiation level. At the end of the five-week period, the diets were switched, and the feeding resumed for five more weeks. Gut mucosal samples were taken for microbiota analysis at week 5 and week 10 from 3 fish per tank. Prior to characterizing the microbial communities of the gut samples, we performed a comparative analysis using both Illumina and Oxford long-read sequencing, targeting the 16S rRNA gene, to understand whether profiling of gut microbial communities is affected by the sequencing platform, and in order to optimize the sequencing conditions. After DNA extraction, gut mucosal samples were sequenced using our optimized protocol for Oxford Nanopore targeting the full-length 16S. Diversity and microbial composition analysis, along with statistical analysis were performed using the microeco R package.

## Results & Discussion

Comparison of different sample types using Illumina MiSeq targeting the V3-V4 region, and long-read sequencing using Oxford Nanopore targeting the full-legth of the 16S rRNA gene, showed some differences in the microbial profiling, especially with relation to the abundance of Proteobacteria and Firmicutes phyla. This has been also previously reported in gilthead seabream microbiota, when performing a similar comparison (Toxqui-Rodríguez et al., 2023). However, when testing whether these differences are affecting the sensitivity of our platform, no significant effects were observed, suggesting that Oxford Nanopore can be used for the characterization of mucosal microbiota.

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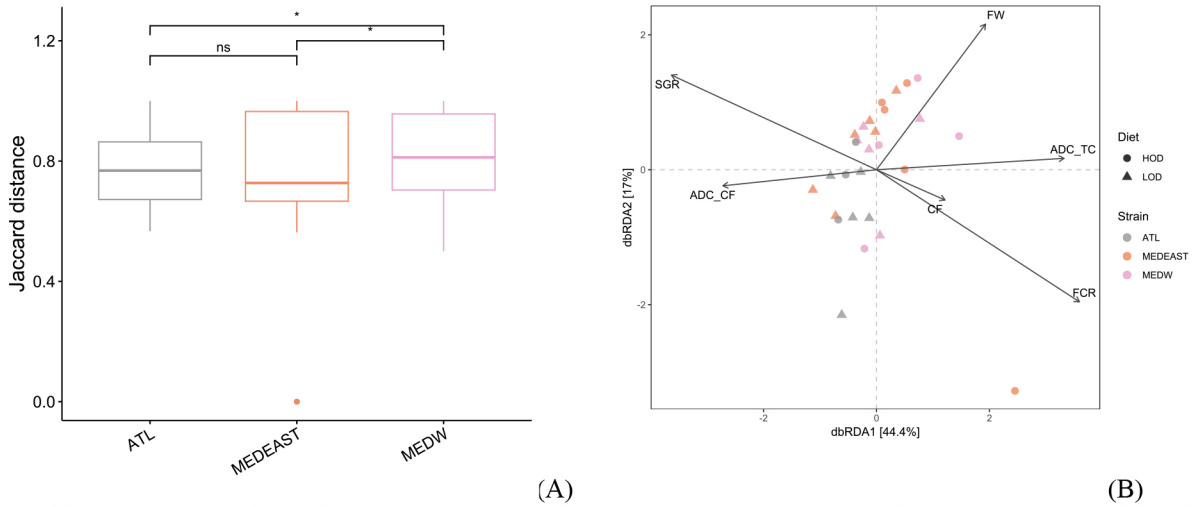


*Fig1. (A) Within group dissimilarity, indicating the level of variance in the microbiota composition of the three European seabass strains. (B) Distance-based redundancy analysis, using growth performance, feed efficiency and digestibility as explanatory variables. SGR, Specific growth rate; FW, final weight; CF, condition factor; FCR, feed conversion ratio; ADC, Apparent Digestibility Coefficient; CF, crude fat; TC, total carbohydrates.*

When comparing the mucosal samples coming from different diets, microbial alpha diversity did not show any significant differences ($P > 0.05$); however, an effect of the strain was found to affect gut microbial structure. Interestingly, we found a higher dissimilarity within the gut microbiota composition of the West Mediterranean strain compared to that of the Atlantic and East Mediterranean strain. Specifically, we found a lower individual variability within the Atlantic strain (Fig1A), which could indicate a more homogenous genetic structure of this strain, making the microbiota of those individuals more similar to each other.

In a similar fashion, a clear separation of Atlantic strain was observed when correlating the structure of microbiota data with growth and digestibility parameters by the distance-based redundancy analysis (dbRDA; Fig1B). The dbRDA plot also may indicate a different capacity of the Atlantic strain in the utilization of different diets. These could be potentially explained by a different coping capacity with dietary changes, and particularly carbohydrate digestion. Similar observations in genetically selected fish were reported by Torecillas et al. (2023), demonstrating capacity to reshape their microbiota to better adapt to the diet.

To conclude, diet did not cause any clear patterns in the gut microbiota diversity of European seabass. However, we observed an effect of the fish strain on the gut microbiota structure and variability, which correlated to different growth rates in the Atlantic strain.

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# SHORT-TERM STORAGE OF RAINBOW TROUT (*Oncorhynchus mykiss*) SPERM: DETERMINATION OF DIFFERENT EXTENDERS, EXTENSION RATES AND IN VITRO CONDITIONS

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## Introduction

Aquaculture is vital for addressing global protein demands, yet broodstock management challenges, particularly in gamete quality, hinder its progress (Liao & Chao, 2009). Short-term sperm storage is a practical technique to synchronize gamete availability in species like rainbow trout (*Oncorhynchus mykiss*), where asynchronous maturation between males and females is common (Mañanós et al., 2009). Sperm quality during storage is influenced by oxidative stress, which can be mitigated by antioxidants such as Vitamin C, Vitamin E, melatonin, and glutathione peroxidase (Shaliutina et al., 2013). This study evaluates the effect of these antioxidants on the quality and fertilization capacity of rainbow trout sperm during short-term storage, comparing broodstocks from their 1st and 3rd spawning years.

## Materials and methods

Sperm samples were collected from rainbow trout broodstocks in their 1st and 3rd spawning years (Year 1 and Year 3). Samples were stored for 28 days at 4°C, either undiluted (fresh sperm) or diluted with extenders containing Artificial Seminal Plasma (ASP), Vitamin C, Vitamin E, Vitamin C+E, Glutathione, Melatonin, or Glutathione + Melatonin. Sperm quality was assessed through motility duration (seconds), live sperm percentage, spermatozoa concentration ($x10^9$/mL), fertilization rates, and morphological characteristics (head and flagellum length). Statistical analyses included ANOVA and Games-Howell Post hoc tests to evaluate differences across treatments, storage days, and spawning years.

## Result and Discussion

Vitamin E significantly enhanced sperm motility across both spawning years, achieving the highest average motility in Year 1 (43.4%) and Year 3 (48.9%), outperforming Vitamin C (23.1% in Year 1, 22.1% in Year 3) and Vitamin C+E (23.4% in Year 1, 23.2% in Year 3) ($p < 0.0001$), likely due to its role in reducing lipid peroxidation (Aitken & Clarkson, 1988). A three-way ANOVA confirmed significant effects of year ($F = 169.792$, $p < 0.0001$), treatment ($F = 274.846$, $p < 0.0001$), and storage day ($F = 7523.168$, $p < 0.0001$) on motility, with Year 3 generally showing higher motility. The Year × Treatment interaction ($F = 17.369$, $p < 0.0001$) indicated varying treatment effects between years, with melatonin improving in Year 3. Figure 1 shows that motility declined over 28 days, with Vitamin E and Artificial Seminal Plasma (ASP) maintaining higher motility longer than Vitamin C and Vitamin C+E. Live sperm percentages also decreased over time, with Vitamin E maintaining the highest viability (60.2% in both years). Fertilization rates dropped to 0% by Day 28, with Year 3 showing higher initial rates (18.56% on Day 0 vs. 16.11% in Year 1), reflecting better baseline quality in older fish (Büyükhatipoglu & Holtz, 1978). Spermatozoa concentration was higher in Year 1 (e.g., 7.43 $x10^9$/mL for fresh sperm) than Year 3 (5.43 $x10^9$/mL) ($p = 0.005$). Morphological analysis revealed larger head lengths in Year 3 (e.g., 3.46 μm for melatonin) and shorter flagellum lengths (e.g., 26.80 μm for Vitamin C) compared to Year 1 ($p < 0.0001$), indicating age-related changes (Billard, 1992). Table 1 summarizes average motility percentages, highlighting Vitamin E's superior performance. These findings suggest Vitamin E is a promising antioxidant for short-term sperm storage in rainbow trout, with age influencing treatment efficacy.

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Table 1. Average Sperm Motility (%) of Rainbow Trout in 1st and 3rd Spawning Years

| Treatments | *Year 1 (%)* | *Year 3 (%)* |
|---|---|---|
| *Fresh Sperm* | 48.9 | 45.4 |
| *ASP* | 46 | 40.5 |
| *Vitamin C* | 45.4 | 22.1 |
| *Vitamin E* | 43.2 | 48.9 |
| *Vitamin C+E* | 40.5 | 23.2 |
| *Glutathione* | 39.6 | 43.2 |
| *Melatonin* | 23.2 | 46 |
| *Glutathione+Melatonin* | 22.1 | 39.6 |

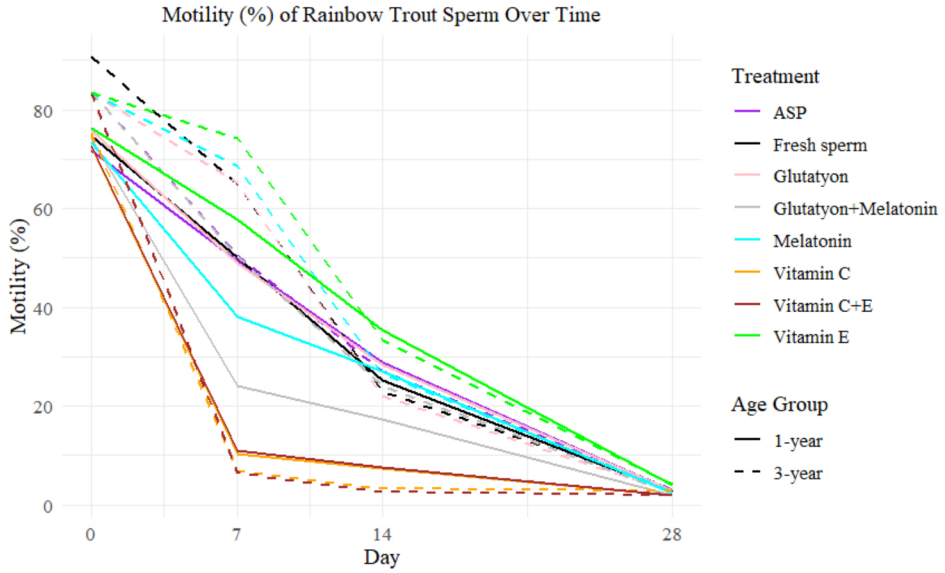


Figure 1: Temporal Changes in Total Sperm Motility (%) of Rainbow Trout (First and Third Spawning Years) Under Different Storage Treatments

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# BARLEY PROTEIN: SUSTAINABLE PERFORMANCE FOR AQUACULTURE

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Barley protein is emerging as a transformative, sustainable alternative to traditional protein sources in aquaculture feeds. Developed through innovative processing techniques that concentrate the protein fraction from the grain, Barley Protein Concentrate (BPC) addresses both the nutritional and environmental challenges facing the industry. Extensive trials have demonstrated that BPC can fully or partially replace fishmeal without compromising growth, feed conversion efficiency, or product quality across a range of commercially relevant species, including Atlantic salmon, Sea bream, Sea bass, Rainbow trout, and Yellowtail.

In large-scale commercial and research settings, BPC-based diets have consistently matched or exceeded the performance of conventional feeds. For example, trout fed diets with significant BPC inclusion (up to 26%) showed equivalent growth rates (SGR 1.43 %/day), feed conversion (1.05) and protein efficiency ratios (2.32), compared to those fed fishmeal-based diets, with some trials indicating improved energy retention and protein digestibility (up to 89%). Similar positive outcomes have been observed in sea bream and yellowtail, confirming the versatility and efficacy of BPC across both freshwater and marine species.

Beyond nutritional performance, BPC offers substantial sustainability benefits. Barley cultivation is less resource-intensive than other major crops, requiring less water and grown typically without irrigation across diverse climates, while the BPC production process eliminating any waste by utilizing all fractions of the grain. By reducing reliance on fishmeal and soy protein-both associated with significant environmental impacts, BPC supports the development of a more resilient and eco-friendly aquaculture sector while delivering excellent growth performance with no antinutrient factors.

This presentation highlights the production processes, testing, and commercial application of barley protein concentrate, underscoring its proven performance and critical role in advancing sustainable aquaculture.

# POLAR LIPIDS IN FEED FOR ATLANTIC SALMON FARMED UNDER ARCTIC CONDITIONS

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## Introduction

Replacing fishmeal and fish oil with plant-based ingredients in Atlantic salmon diets has led to concerns regarding gut health and welfare, including excessive lipid accumulation (intestinal steatosis) and inflammatory reactions. Some of these issues are likely linked to altered nutrient profiles, including low choline levels in plant ingredients. Choline is critical for lipid transport, membrane structure, and methylation, and its deficiency is strongly associated with intestinal steatosis in salmon. Studies have shown that dietary choline, particularly as phosphatidylcholine or choline chloride, can prevent such symptoms [1]. Factors such as high dietary fat, warmer water temperatures, and rapid growth increase choline demand, especially in Arctic farming conditions where steatosis prevalence shows large seasonal variations. Data from the Arctic Salmon Research Centre (2016–2024) show a marked increase in steatosis during September–December, i.e. during the warmest period with high growth and feed intake. The current study aimed to assess the effect of krill meal - rich in phosphatidylcholine - on intestinal steatosis, mucosal health in gut and skin, flesh quality, and overall performance of Atlantic salmon farmed under Arctic conditions. It also sought to determine whether krill-derived choline differs from choline chloride in preventing steatosis, potentially supporting more targeted supplementation strategies for enhanced health and production outcomes.

## Materials and methods

The trial was conducted at the Cermaq farming site at Storholmen (Bekkarfjorden, Finnmark, 70°20′09″N 23°19′36″E) using 9 open 120 $m^2$ sea cages, each stocked with 200,000 smolts averaging 108 g in Aug 2023. The trial started when fish reached an average estimated body weight of 300 g in Oct 2023. The fish were fed either a commercial diet with total choline supplied from 15% fishmeal only (Ctr) or a diet with high total choline from 10% krill meal + 5% of fishmeal (KM) or a diet with high total choline similar to KM diet from a combination of 15% fishmeal and choline chloride supplementation, respectively (ChCl), in triplicate cages each. Fish were fed Ctr and ChCl diets throughout the trial period (Oct 2023-Dec 2024) while fish were fed KM diet from Oct 2023-May 2024 and then continued to be fed the Ctr diet for rest of the trial period. All three diets contained similar gross composition, designed to meet the known nutritional requirements of Atlantic salmon. Pellet size and dietary composition were adjusted to fish size. Fish were initially sampled in Oct 2023 after being fed a commercial diet and subsequently at four sampling time points in Dec 2023, Apr, Sep, and Dec 2024, after feeding the experimental diets. Possible effects of the diets on performance, general morphology, fish welfare, gut- and skin health, nutritional status and sensory quality were evaluated by a comprehensive analytical package.

## Results and discussion

The fish grew from an initial body weight of 0.3 kg to an overall average body weight of 3.8 kg during the feeding trial, corresponding with an overall thermal growth coefficient of 3.44 from Oct 2023-Jan 2024. The body weight of the fish was affected by both the diet and time. Fish fed the diet supplemented with choline from krill meal (KM) as main source or fish meal and choline chloride (ChCl) in the diet showed generally lower growth compared to the fish fed the control diet with choline supplied only from fish meal. The final weights of Ctr, KM and ChCl groups were 4.1, 3.6 and 3.7 kg respectively in the last sampling point in Dec 2024. Feed conversion ratio and average mortalities for the trial period did not differ significantly among diet groups. Average mortalities were 3.8% in Ctr, 4.1% in KM and 3.5% in ChCl groups, mainly due to infections caused by *Parvicapsula pseudobranchicola*.

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Occurrence of skin ulcers was low across the feeding groups, with no significant difference due to dietary treatment. On the contrary, supplementation of KM or ChCl showed indications of reduced welfare in terms of increased scale loss (ChCl), eye bleeding and higher scores for red belly (both ChCl and KM) in Sept 2024. Skin health, as evaluated by blood plasma antibody levels, skin morphometrics and transcriptomics showed generally few clear effects of diet. KM supplementation seemed to enhance the integumentary health of Atlantic salmon; however, this improvement was not sustained after supplementation ended.

With regards to sensory quality, high inclusion level of choline from fish meal and choline chloride showed positive effects on fillet yield, however there was no effect on fillet color. Some negative effects with reference to melanin deposition, gaping, and texture, were observed. The fatty acid composition of the muscle generally reflected the type of feed treatment for each group, which was expected.

The prevalence and severity of intestinal steatosis symptoms were relatively low in all three diet groups during the period Oct 2023-Sept 2024, indicating that the choline requirement was met for most of the fish during this period. In contrast, moderate to marked intestinal steatosis was observed in most of the fish at the last sampling time point in Dec 2024. No statistically significant differences among diet groups were registered, but there was a trend towards ChCl-fed fish (having the highest dietary choline levels) displaying less severe steatosis symptoms. In this regard, it should be mentioned that analysed choline levels varied less than originally formulated between Ctr, KM, and ChCl fish groups. Dietary choline levels in the diets that were fed from April 2024 and throughout the remaining part of the trial, were 3.1 and 3.4 g/kg for the Ctr and ChCl diets, respectively. These levels fall within the range of the estimated choline requirement of 3.4 g/kg for post-smolt salmon of about 0.5 kg when fed a diet with 29% lipid [1]. However, the lipid level of the current diets was higher, about 34%. As increasing dietary lipid level has been shown to increase severity of steatosis, and thereby the choline requirement [2], it is possible that the choline requirement was not fully met in the latter part of the present trial. Further studies with greater contrasts in dietary choline levels are needed to better understand choline requirements in salmon under varying production conditions.

Overall, this study found that 11% inclusion of krill meal replacing fish meal from a diet containing 16% fish meal had no positive effect on growth, feed utilization, overall mortality and welfare, skin and gut mucosal health, or fillet quality.

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# CHARACTERIZATION OF POLY(3-HYDROXYBUTYRATE) AND DEVELOPMENT OF BIOGENIC AND BIODEGRADABLE MATERIALS FOR PACKAGING AND SHELF LIFE MONITORING OF SEABREAM FILLET (*Sparus aurata*)

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## Introduction

The widespread use of conventional plastics in food packaging has led to major environmental concerns due to their non-renewable origin, persistence, limited biodegradability and inefficient waste management (Bucci et al., 2005; North & Halden, 2013). In response, there is a growing focus on sustainable alternatives such as bioplastics - biodegradable material derived from renewable sources - aiming to reduce the environmental footprint and support circular economy goals (Joseph et al., 2023; Rosenboom et al., 2022). In addition, the food industry is confronted with the urgent challenge of minimizing food waste while maintaining food safety and quality (Uysal-Unalan et al., 2024). Fish are among the variety of food products demanding effective packaging solutions (Lionetto & Esposito Corcione, 2021). Therefore, this study investigated the development of plasticized poly(3-hydroxybutyrate) (PHB) films for application as sealing layers in the packaging of sea bream (*Sparus aurata*) fillets. The objective was to assess their performance in microbial load control and propose a novel, environmentally sustainable packaging solution that preserves the quality of the product.

## Materials and methods

The films produced via the solvent casting method were prepared from film-forming solutions (FFS), using PHB dissolved in chloroform, with the addition of plasticizers such as polyethylene glycol (PEG 200, 3350, 8000). A series of preliminary experiments optimized key parameters including polymer concentration (8–10% w/v), solvent type, temperature and mixing time, to achieve homogeneous solutions and films with suitable thickness and integrity. Final films were standardized to 80-100μm thickness using a precision applicator and evaluated for their mechanical (tensile strength, elongation at break, Young's modulus), optical (CIELab color, UV-Vis light transmittance, opacity) and barrier (water vapor transmission rate) properties. Additional analyses included swelling behavior in aqueous media, surface wettability (contact angle), and surface free energy (SFE), providing a comprehensive assessment of the material's performance. The film with the most desired mechanical properties was selected and placed as a sealing layer in the packaging of sea bream (*Sparus aurata*) fillets. Microbiological tests were performed to monitor microbial spoilage in fillets stored under controlled refrigeration conditions (4°C) for shelf life evaluation.

## Results and Discussion

The investigation into PHB film production revealed that polymer and plasticizer concentration, stirring/heating duration, and casting temperature significantly influenced the films' final properties. The desired formulation was achieved by dissolving 8% w/v PHB with 20% w/w PEG200 in chloroform under prolonged stirring near the solvent's boiling point, followed by solvent casting at 60ºC. This composition yielded films with superior mechanical stability, high elongation at break, and a uniform appearance. PEG3350 and PEG8000 showed poorer compatibility and performance, with PEG8000 also presenting miscibility issues at higher concentrations. The selected formulation (C20PEG200) exhibited favorable visual and UV-Vis barrier properties but showed limitations in water contact, including increased hydrophilicity, water-induced shrinkage, and high water vapor permeability. Microbial spoilage and sensory degradation in fish fillets demonstrated that PHB-based films can serve as alternative packaging materials for fresh fish fillets, without compromising quality and shelf life. The shelf life of gilthead seabream fillets was estimated at 5-6 days at 4 °C for all tested packaging materials.

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# EFFECT OF DIETARY PHOSPHORUS AND CALCIUM LEVELS ON BONE DEVELOPMENT OF ZEBRAFISH

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**Introduction**

Phosphorus (P) plays a vital role in fish diets, contributing to bone formation and mineralization (Lall, 2002). Zebrafish (*Danio rerio*) is an established model in nutritional research (Antinero et al., 2023, Molinari et al., 2025) and is responsive to the same factors known to cause deformities in farmed fish (Costa et al., 2018; Printzi et al., 2024). Previous studies have shown that dietary P influences bone mineralization in zebrafish and other species post-metamorphosis i.e., after the development of the skeleton has been completed (Witten et al., 2019; Cotti et al., 2020). However, little is known about its impact during the early larval period, i.e., during the development of zebrafish skeleton. This study investigates how varying dietary phosphorus and calcium levels affect skeletal development, mineralization patterns, and deformities in zebrafish larvae and the the post-metamorphosis skeleton, with the aim of improving larval nutrition and skeletal health.

**Materials and methods**

To evaluate the effects of dietary phosphorus (P) and calcium (Ca) on zebrafish larval skeletal development, three experimental diets with varying P/Ca levels were formulated: Diet A (3.20% P / 1.67% Ca), Diet C (2.20% P / 1.90% Ca), and Diet G (2.22% P / 1.62% Ca). A commercial zebrafish feed (Zebrafeed, Sparos) served as a control. Skeletal quality was assessed at approximately 7 mm and 10 mm total length (TL) by analyzing vertebral column mineralization and skeletal abnormalities, respectively. At 10–11 mm TL, fish underwent a swimming challenge test (SCT) to evaluate vertebral resilience against haemal lordosis.

**Results and discussion**

The mineralization rate of the vertebral column was significantly influenced by dietary phosphorus and calcium during the critical growth window of 5.5–7.5 mm total length (TL) (ANOVA, $p < 0.05$). Fish under regime G (low P / low Ca) exhibited significantly lower centra mineralization (6–7 mm TL: 12.5 ± 11.9; 7–8 mm TL: 24 ± 9.9) than those in regimes A (high P / low Ca), C (low P / high Ca), and N (control), which all showed similarly higher values (e.g., A: 6–7 mm TL: 25.2 ± 10.6; 7–8 mm TL: 30.7 ± 1.2).

Skeletal deformities were significantly influenced by diet ($p < 0.05$). Regime A (high P / low Ca) showed the lowest rates of light kyphosis (10 ± 6%), scoliosis (7 ± 6%), resorption (1 ± 1%), and branchiostegal ray twisting (41 ± 5%). These rates increased in regime N (control) (25–57%), were higher in C (42–59%), and peaked in G (low P / low Ca) with 34%, 47%, 28%, and 77% respectively.

Fish from regime A exhibited the lowest overall incidence of haemal lordosis (74.7%), significantly lower than those from regimes C and G (92.2% and 91.4%, respectively). In severe cases, only 15.7% of regime A individuals were affected with significantly higher 57.8% and 45.7% in regimes C and G, respectively.

Overall, our results demonstrate that increased dietary phosphorus significantly enhanced skeletal development, mineralization, and deformity resistance in zebrafish larvae while higher dietary calcium levels partly compensated for low phosphorus.

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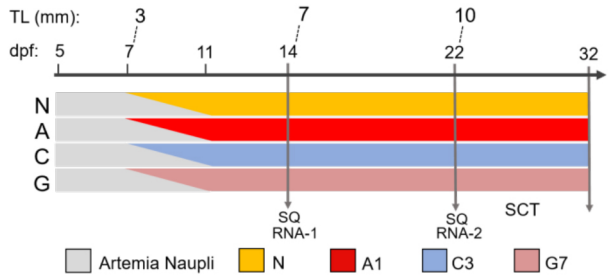


Figure 1. The feeding regime followed Printzi et al., (2021). Samplings for larval skeletal quality (SQ) took place at early (14 dpf) and late larval stages (22 dpf). The swimming challenge test (SCT) took place after the last sampling. Trials were performed in triplicate. Dpf, days post fertilization.

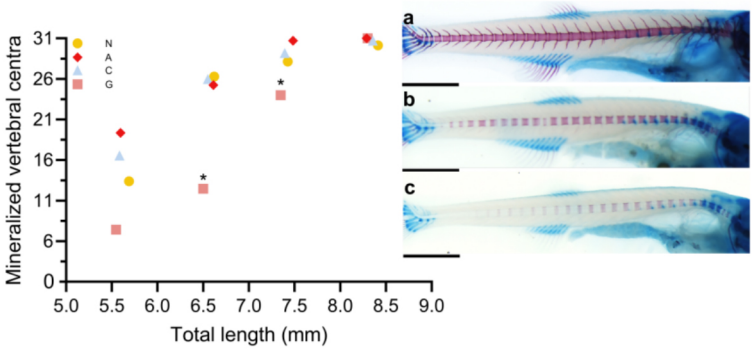


**Figure 2.** Effect of dietary components on the mean number of mineralized vertebral centra at 14 dpf. Significant differences are indicated by the asterisk (*) ($p<0.05$). (a-c) Representative samples for each regime of the same length (7.2 mm TL) but with different mineralization status. a) Regime A b) Regimes N and C, and c) Regime G.

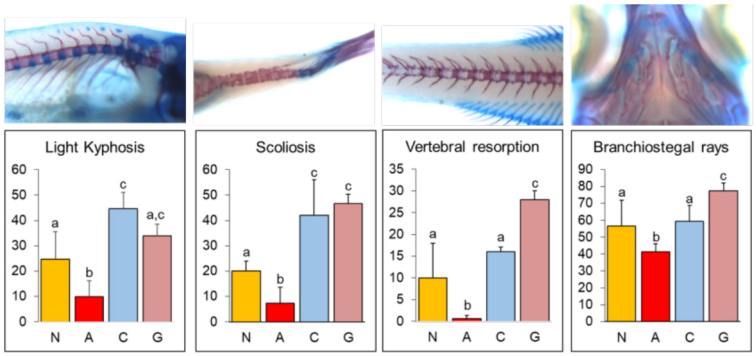


**Figure 3.** (a-g) Effect of dietary components on the frequency (±SE) of skeletal abnormalities in zebrafish larvae at 22 dpf. (a) Light kyphosis. (b) Scoliosis. (c) vertebral resorption (d) twisted branchiostegal rays.

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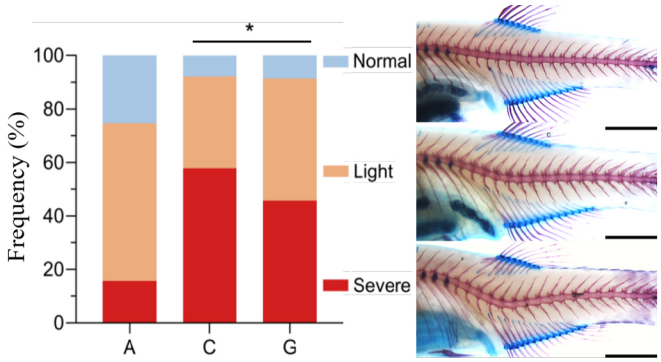


**Figure 4.** Proportion (%) of normal, light and severe lordotic fish per each diet.

Overall, our results demonstrate that increased dietary phosphorus significantly enhanced skeletal development, mineralization, and deformity resistance in zebrafish larvae while higher dietary calcium levels partly compensated for low phosphorus.

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# INVESTIGATING HYPOTONIC EXTENDER-BASED CRYOMEDIUM FOR STURGEON SPERM CRYOPRESERVATION

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**Introduction**

Sturgeons (Acipenseriformes) are ecologically and economically significant fish facing severe population declines, necessitating advanced conservation strategies. Sperm cryopreservation is critical for ex-situ genetic preservation and enhancing aquaculture productivity through artificial fertilization. Developing effective cryopreservation protocols requires understanding the unique characteristics of sturgeon spermatozoa, particularly their dependence on potassium ions (K+), which are critical for inhibiting motility. We hypothesized that a hypotonic extender containing only 1 mM KCl could effectively inhibit sperm motility and support successful cryopreservation, offering an alternative approach compared to standard sturgeon protocols.

**Materials and methods**

This study evaluated sperm cryopreservation protocols across sterlet (*Acipenser ruthenus*), Siberian (*A. baerii*), Russian (*A. gueldenstaedtii*), and stellate (*A. stellatus*) sturgeons, comparing a novel cryomedium (1 mM KCl, 11% methanol, 2.5% ethylene glycol) against a traditional cryomedium. Two cryopreservation formats were tested: 0.5 ml straws with rapid cooling on a polystyrene raft in a Styrofoam box, and 4.5 ml and 10 ml cryotubes with slow, controlled cooling in a programmable freezer. Post-thaw motility, spermatozoa volume changes during equilibration, fertilization success, and embryogenesis were assessed.

**Results**

The hypotonic cryomedium preserved high post-thaw motility: Siberian sturgeon achieved 46±6% (0.5 ml straws and 4.5 ml cryotubes); Russian sturgeon, 31±10% (0.5 ml straws) and 40±12% (10 ml cryotubes); and stellate sturgeon, 46±10% (0.5 ml straws), 39±8% (4.5 ml cryotubes), and 44±11% (10 ml cryotubes). Fertilization and hatching rates were robust, with Siberian sturgeon reaching 81±16% and 79±15% (0.5 ml straws), and 84±6% and 82±7% (4.5 ml cryotubes), and Russian sturgeon achieving 60±20% and 57±20% (0.5 ml straws), and 62±11% and 48±13% (10 ml cryotubes). Ethylene glycol induced initial sperm cell shrinkage followed by volume normalization, unlike methanol, which caused minimal volume changes. Although curvilinear velocity (VCL) was significantly reduced in the hypotonic cryomedium compared to fresh sperm and traditional cryomedium ($p<0.05$), fertilization success remained unaffected, and embryos showed no significant morphological abnormalities.

**Conclusion**

This study confirms the effectiveness of the 1 mM KCl-based hypotonic cryomedium for cryopreserving sperm across multiple sturgeon species, and sample volumes, supporting both small-scale (0.5 ml straws) and large-scale (4.5–10 ml cryotubes) cryopreservation without compromising reproductive outcomes. This protocol offers a versatile, scalable approach for sturgeon sperm cryopreservation, advancing conservation and aquaculture practices by facilitating large-scale breeding.

# DO YOU KNOW YOUR SYSTEM'S MICROBIOME? – MICROBIOME MONITORING AS A KEY MANAGEMENT TOOL FOR AQUATIC ANIMAL HEALTH

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The microbiome plays a key role in maintaining health and ecological stability in aquatic systems, especially in closed and controlled environments such as recirculating aquaculture systems (RAS), aquaria and other closed aquatic environments. Recent progress in microbial research and molecular analysis has exposed the intricacies and variability of these communities, highlighting their influence on water quality, pathogen control and the overall well-being of aquatic animals.

Despite its importance, microbiome monitoring has rarely been integrated into routine husbandry, largely due to the lack of accessible tools. This project presents a newly developed, user-friendly test kit that allows microbiome composition in keeping systems to be assessed in a practical, standardized and reliable way. By analyzing bacterial community diversity, the tool enables the early detection of microbial imbalances and facilitates informed intervention. Case studies from both private aquaria and professional facilities demonstrate that routine microbiome monitoring can lead to measurable improvements in water quality and animal health.

Integrating microbiome diagnostics into regular husbandry offers significant potential for research, conservation and education, as well as operational benefits. Standardized sampling protocols and the sharing of microbial data across institutions could facilitate large-scale comparative studies and advance our understanding of aquatic microbiomes. This could also support the development of best practice guidelines.

Our test kit promotes the wider use of microbiome-based diagnostics as a preventive management tool based on evidence, connecting cutting-edge microbial science with practical aquaculture health management.

# EFFECT OF ELEVATED CARBON DIOXIDE ON GROWTH PERFORMANCE AND SOMATIC PARAMETERS IN RAS-BASED JUVENILE PIKEPERCH (*Sander lucioperca*)

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## Introduction

In recirculating aquaculture systems (RAS), carbon dioxide ($CO_2$) accumulates due to respiration and microbial activity, potentially impairing fish growth performance. Pikeperch (*Sander lucioperca*) is a promising candidate for intensive aquaculture in Europe (Müller-Belecke and Zienert, 2008; Overton et al., 2015; Policar et al., 2019), yet limited information is available regarding its tolerance to elevated $CO_2$ levels (Skov, 2019). This study aimed to evaluate the effects of three dissolved $CO_2$concentrations (0, 15, and 30 mg/L) on the growth, feed efficiency, and physiological condition of juvenile pikeperch over a rearing period. Understanding $CO_2$ related responses is essential to optimize water quality management and welfare in intensive RAS.

## Materials and Methods

Juvenile pikeperch (initial total length ~254 mm, body weight ~117 g) were randomly assigned to one of three $CO_2$ treatments: Low (0 mg/L), Medium (15 mg/L), and High (30 mg/L). Each treatment was replicated in triplicate tanks (50 fish per tank) connected to a shared RAS with controlled temperature, oxygen, and photoperiod. Fish were reared for 112 days and fed a commercial diet to apparent satiation by belt feeders. Key performance parameters, including total length (TL), standard length (SL), body weight (BW), and Fulton's condition factor (K). Fish were measured at the beginning and end of the trial. Feed conversion ratio (FCR), specific growth rate (SGR), thermal growth coefficient (TGC), and survival rate (S) were calculated. Additionally, organosomatic indices (HSI, FSI, GSI, KSI) were determined at the end of the experiment. Fin erosion among tested groups was assessed at the beginning and end of the experiment. Variation in body weight was assessed using coefficient of variation (CVBW). Differences between treatments were analyzed by one-way ANOVA with Tukey's post-hoc test ($\alpha$ = 0.05). The assessment of the pectoral, ventral, dorsal, caudal, and anal fins, along with the rate of fin erosion, was conducted based on the methodology described by Policar et al. (2016).

## Results

Initial biometric data showed no significant differences among treatment groups ($P > 0.05$). At the end of the trial, fish reared under high $CO_2$ (30 mg/L) had significantly lower TL, SL, BW, and K compared to the low $CO_2$ group ($P < 0.05$). Final BW was highest in the low $CO_2$ group (297.34 ± 69.60 g), compared to medium (250.02 ± 69.62 g) and high (232.93 ± 60.81 g) $CO_2$. Feed efficiency was negatively affected at higher $CO_2$ concentrations, with FCR increasing from 1.09 (low $CO_2$) to 1.82 (high $CO_2$). SGR and TGC decreased with increasing $CO_2$, though differences were marginally non-significant ($P = 0.063$ and $P = 0.066$, respectively). No significant differences were observed in survival, organosomatic indices, or CVBW. No differences in fin erosion were observed between the tested groups.

## Discussion

Elevated $CO_2$ concentrations in RAS significantly reduced the growth and condition of pikeperch, likely due to increased metabolic costs and reduced feed efficiency. These findings suggest that $CO_2$ concentrations above 15 mg/L may compromise fish performance, despite not affecting survival or physiological indices. Maintaining lower $CO_2$ levels appears beneficial for maximizing growth and feed efficiency in intensive pikeperch culture. The study highlights the need for $CO_2$ control in RAS design and operation to ensure optimal fish welfare and production outcomes.

*(Continued on next page)*

**Acknowledgements**
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# ASSESSEMENT OF CHARACTERISTICS OF SOME PLANT VARIETIES (BASIL, MULLEIN, AND ROSEMARY) ON GOLDFISH (*Carassius auratus*)

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**Introduction**

Nowadays, anesthetics are the important substances in aquaculture applications. They are consist of two kinds, chemicals and herbals. In our research, 3 different anesthetic plants were used in order to see the efficiency of them to goldfish. Actually, anesthesia is virtual things to eleviate stress reactions and injury damages during handling procedures (measuring weighing, grading, tagging, vacination, transport, blood sampling, biopsies of gonads, gamet collection, etc.) in aquaculture (Coyle et al., 2004; Mylonas et al., 2005). Several anesthetics are commonly used for many differnt fish species such as tricaine metansulfonate (MS-222), phenoxyethanol, quinaldin, benzocain, clove oil and lavander (Mercy et al., 2013). There is a lot of considerations to decide which anesthetics is suitable for which species of fish. In this study, the anesthetic effects of three different herbal anaesthetics (basil, mullein and rosemary) on goldfish were investigated.

**Materials and amethods**

Basil was perchased and mullein and rosemary were collected from located area. Then, they were dried in the laboratory. Then dried plants were grained and each powdered plant samples were added to 100 g, 200 g ve 300 g to 1.5 L distiled water. Each mixture was mix a time a day within 3 days in the frige +4 ºC condition and saved and plant solutions were prepared. Then, they were used to prepare the concentrations of 30, 40, 50, 60, and 70 ml/L for each plant. Induction and recovery times were indicated and saved for those concentrations. For each concentration, 5-10 fish were used and totally 225-450 fish were used. These anesthetic efficiency of 3 herbal plant species were researhed to evaluate for aquaculture applications.

**Results**

For 100 g, 200 g, 300 g of rosemary plant dose and 30-70 ml/L concentrations were formed 0.16-0.98 in I1, 1.90-5.02, in I2, 0.20-1.29 in R1 and 1.53-3.47 in R2. For 100 g, 200 g, 300 g ofmullein plant dose and 30-70 ml/L concentrations were found as 0.14-0.55 in I1, 3.07-8.18 in I2, 0.22-2.84 in R1 and 1.26-6.58 in R2. For 100 g, 200 g 300 g of basil plant döşe and 30-70 ml/L concentrations were saved as 0.15-0.73 in I1, 1.29-6.23 in I2, 0.31-1.33in R1 and 1.32-3.59 in R2.

Herbal anesthetic usege caused some histological disorders in goldfish in this research. In rosemary usage, hyperplasia, degenerations; in mullein usage, fusion, hyperplasia and edemma; in basil usage, hyperplasia, and aneurism were seen in gills. In rosemary usage, degenerations in cell walls, necrotic structure formation, granulomatic structure developments; in mullein usage, valuolations, picnotic nuclei, necrotic structure formation; in basil usage, picnotic nuclei, necrotic structure formation, and granulomatic structure developments were dedected in liver. In rosemary usage, renal tubular vacuolations, renal tubular degenerations, and granulomatic structure developments; in mullein usage, renal tubular vacuolations, granulomatic structure developments and renal tubular degenerations; in basil usage, renal tubular vacuolations, renal tubular degenerations, granulomatic structure developments were occured in kidney.

# APPLICATION OF LOW-COVERAGE WHOLE GENOME SEQUENCING FOR UNCOVERING THE GENETIC BASIS OF FERTILITY IN ARCTIC CHARR (*Salvelinus alpinus*)

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## Introduction

Arctic charr (*Salvelinus alpinus*) is a key cold-water salmonid for aquaculture in the Nordic countries (Olk et al., 2020) and a species of high ecological (Jónsdóttir et al., 2024) and evolutionary interest (Kess et al., 2021; Kurta et al., 2025; Skoglund et al., 2015). In Sweden, the national Arctic charr breeding programme was initiated over 40 years ago and continues to deliver consistent genetic gains of about 10% per generation (Carlberg et al., 2018). While the programme has successfully improved growth performance through selective breeding (Nilsson et al., 2010), fertility and other reproductive traits have received relatively little attention, despite their critical importance for production efficiency. Sperm quality traits such as concentration and motility have been reported as important bioindicators of male fertility in fish (Gallego et al., 2013) and livestock (Ramirez-Diaz et al., 2023), and are therefore of high interest in genetic studies.

Whole genome sequencing (WGS) has been shown to successfully reveal fine-scale, "cryptic" genomic variation that may not be detected with limited marker sets (Andersson et al., 2024). Low-coverage whole genome sequencing (lcWGS) offers a cost-efficient way to genotype hundreds to thousands of individuals at genome-wide scale. This approach enables the discovery of variants associated with complex traits, and characterization of population-genetic structure in both wild and farmed stocks.

This study focused on applying lcWGS to identify genomic regions linked to sperm quality traits in Swedish Arctic charr. By aligning the sequencing reads to a newly assembled reference genome developed for the same selectively bred Arctic charr strain, we aim to improve mapping accuracy and enhance the resolution of detected fertility-associated loci, strengthening the genomic resources available for selective breeding.

## Materials and methods

At this stage of the study, 150 selectively bred males were sequenced and phenotyped for sperm concentration, motility, and swimming velocity, forming the basis for a pilot genome-wide association analysis. These individuals represent a subset of a larger phenotyped cohort of ~1,000 fish, providing substantial scope for future expansion of the genomic dataset. At the first stage of the study, individual whole genome sequencing libraries were prepared for n = 150 DNA sample using a custom Tn5-based protocol (Picelli et al., 2014) and pooled for sequencing on Illumina NovaSeq S4 lanes. The obtained reads were aligned to newly assembled reference genome available for the Swedish selectively bred Arctic charr. Low-coverage (1-2×) sequencing data were processed using ANGSD v0.933, a toolkit specifically designed for genotype likelihood estimation from low-coverage sequencing data. Genotype likelihoods were used to estimate population-specific allele frequencies and assess the population structure. In addition, a genome-wide association study (GWAS) was performed to identify genomic regions associated with sperm quality traits in the selectively bred Arctic charr strain.

## Results

Preliminary QC metrics indicated consistent sequencing depth and uniform genome coverage across individuals, enabling reliable estimation of genotype likelihoods. We further expect to identify genome-wide regions associated with sperm quality traits that could not be detected using smaller SNP panels (Kurta et al., 2022) or limited sample size (Kurta et al., 2023). Analyses will also leverage a newly available, high-quality reference genome for selectively bred Arctic charr, increasing mapping accuracy and enabling finer resolution of candidate regions. These findings will provide valuable insights into the genomic architecture of male fertility in Arctic charr and support the integration of fertility traits into ongoing selective breeding programmes.

## Acknowledgements

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# REAL-TIME MONITORING OF OCCURRANCE OF PLANKTONIC SEA LICE (*Lepeophtheirus salmonis*) STAGES IN LICE TRAPS USING MACHINE LEARNING

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**Introduction**

Sea lice has for many years been a headache and a major cost driver for the Atlantic salmon (*Salmo salar* L.) farming industry, and many different technologies for removing the sea lice on infected salmon have been developed in the last decades. Blue Lice is a start-up company which has developed a system for capturing the free-swimming sea lice life stages before they are able to attach to their host by using specific illumination signals to attract and capture them via pumps and filters. The system is currently deployed at several sea farms, but although the farmers claim the technology has a positive effect on the sea lice levels, , the actual effect has still not been quantified and documented due to the many challenges related to conducting research in full-scale field trials. In order to assess how effective Blue Lice's technology is in capturing free-swimming sea lice at salmon sea farms, we developed a system for counting the number of captured sea lice in real-time using camera systems and machine learning.

**Materials and method**

We present a high-speed flow-through imaging system (measuring 400mm x 200mm x 130mm) capable of acquiring sharp, high-resolution (22 MP) images of small particles (0.2 mm x 0.2 mm) passing through a flow cell with sea water passing through at velocities up to 0.5 m/s, the system is capable of imaging and processing 14L of water per minute. Built into this system is a state-of-the-art edge computer (Nivida Jetson AGX Orin) for real-time processing of the imaging data and a 4G modem for automatically transmitting the results to the cloud for real-time visualization of the estimated amount of sea lice captured by the Blue Lice technology.

The technology includes a water filtering system that automatically cleans the filters by reversing the flow direction through the filter, re-directing it to a buffer tank where sea lice and other particles with the seawater can pour through to an interchangeable filter bag, essentially up concentrating the seawater. The system is capable of pumping 900 L/m, meaning when implementing the flow-through imaging system between the buffer tank and the interchangeable filter (see Fig. 1), we are also capable of imaging and processing 900 L/m.

**Results**

So far, a prototype has been thoroughly tested in a lab environment at SINTEF SeaLab in over 200 simulated filter cleaning operations. In this process, we have gathered over 150.000 images of seawater containing salmon louse copepodids and nauplii, copepods (*Acartia tonsa*) and preserved samples collected from the Blue Lice technology deployed at a fish farm. The data has been used to train a neural net (FCN; Fully Convolutional Network) to automatically detect sea lice (Fig. 2).

**Conclusions**

So far, the system has been proven accurate for detecting and counting sea lice in a simulated lab setup. However, when it is implemented in a real-world scenario, we expect the image data to be very different, with higher biodiversity and changes between seasons. Using the 4G modem, we can upload image data while the system is in the field so that we are able to improve the detection algorithm and training data on the go, increasing the systems capability and accuracy over time. This system can also, with time, detect other species, giving it a use-case beyond only counting sea lice.

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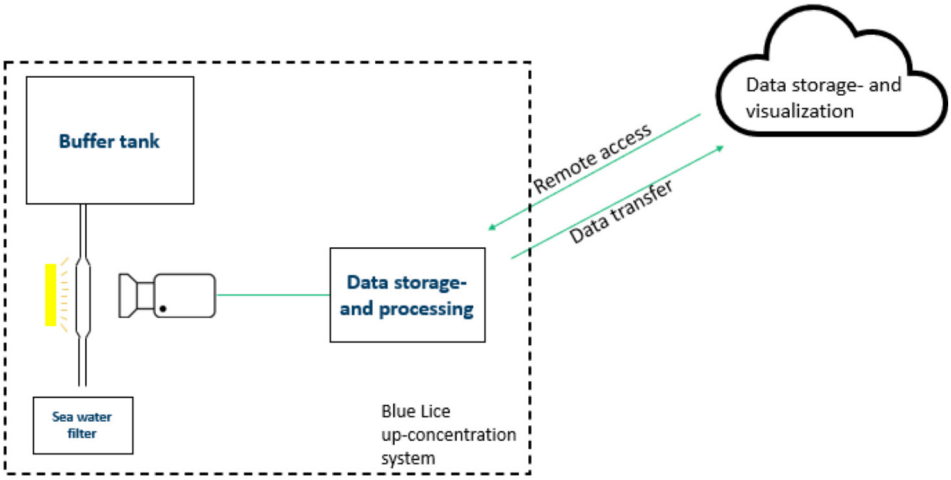


*Figure 1. A concept of the flow-trough system integrated in the Blue Lice technology*

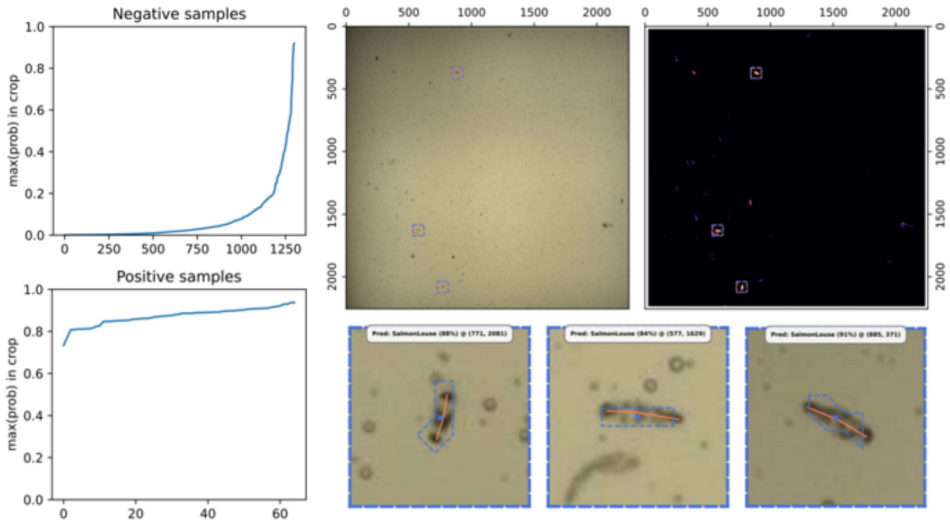


*Figure 2. Detection probabilities of non-lice particles (upper left), detection probablities of salmon lice (lower left), input- and output image from the neural network (upper right), and crops of detected lice (lower right).*

# DIETARY SUBSTITUTION EFFECT OF FISH MEAL WITH PROCESSED POLLOCK BY-PRODUCT MEAL ON GROWTH PERFORMANCE OF OLIVE FLOUNDER (*Paralichthys olivaceus*), AND ECONOMIC ANALYSIS

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## Introduction

The rising price and limited availability of fish meal (FM) is driving the search for an alternative protein source to ensure sustainable aquaculture (Hussain et al., 2024). Pollock by-product meal (PPBM) is one of fishery by-products after processing dried fish. It is mainly composed of dried head, fin, skin, and flesh remaining after manufacturing process of the dried pollock. This study aims to evaluate dietary substitution effect of FM with PPBM on growth of olive flounder (*P. olivaceus*), and economic analysis.

## Materials and Methods

A total of 600 juvenile (6.48 ± 0.016 g; mean ± SEM) fish were randomly assigned to 15, 300-L flow-through tanks (40 fish/tank) in triplicate. All experimental diets were formulated to be isoproteic (51.0%) and isolipidic (12.0%). The control (Con) diet contained 60% FM. In the Con diet, 20%, 40%, 60%, and 80% of FM were substituted with PPBM, referred to as the PPBM20, PPBM40, PPBM60, and PPBM80 diets, respectively (Table 1). Fish were hand-fed to apparent satiation twice a day for 50 days. The formula of economic profit index (EPI) was employed by Montenegro et al. (2022)'s study. One-way ANOVA and Duncan's multiple range test were used.

## Results

At the end of the 50-day feeding trial, weight gain (WG) and specific growth rate (SGR) of olive flounder fed the Con, PPBM20, and PPBM40 diets were significantly ($P < 0.0001$ for both) higher than those of fish fed the PPBM60 and PPBM80 diets (Fig. 1).

## Conclusion

In conclusion, up to 40% of FM could be substituted with PPBM in the 60% FM-based diet without significantly negative effects on the growth performance of olive flounder, and EPI.

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# INCLUSION EFFECT OF VARIOUS LEVELS OF JACK MACKEREL MEAL IN OLIVE FLOUNDER (*Paralichthys olivaceus*) DIETS SUBSTITUTING 50% OF FISH MEAL WITH CHICKEN BY-PRODUCT MEAL ON THE GROWTH PERFORMANCE, FEED UTILIZATION, AND ECONOMIC ANALYSIS

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## Introduction

The low-fish meal (FM) diets often lead to deteriorated palatability, reduced feed consumption, and lowered growth performance of fish. Incorporating feed enhancer or feed ingredient exhibiting strong feed enhancer response to a target fish into the low-FM diets could improve their palatability and feed consumption by fish. Jack mackerel meal (JMM) has been reported to exhibit the strongest attractiveness to olive flounder among 15 protein ingredient sources (Jeong et al., 2022) and its inclusion in the low-FM diets substituting various levels of FM with animal protein sources could improve feed consumption and growth performance of olive flounder (Islam et al., 2024 a). This study aims to evaluate the inclusion effect of various levels of jack mackerel meal in diets substituting 50% of FM with chicken by-product meal (CBM) on the growth performance and feed utilization of olive flounder (*Paralichthys olivaceus*), and economic analysis.

## Materials and Methods

Seven experimental diets with isoproteic (55.0%) and isolipidic (12.0%) were prepared (Table 1). In the control (Con) diet, 60% FM was included. In the Con diet, 50% of FM was substituted with CBM, and then 0%, 10%, 20%, 30%, 40%, and 50% levels of JMM were included at the expense of FM, referred to as the CJ0, CJ10, CJ20, CJ30, CJ40, and CJ50 diets, respectively. A total of 525 juvenile olive flounder (initial mean weight of fish of 12.1g) were distributed into 21, 50-L flow-through tanks (25 fish/tank) with triplicate. All fish were hand-fed to apparent satiation twice a day for 8 weeks. All chemical composition was analyzed according to AOAC methods (1990). Also, the economic analysis was conducted. One-way ANOVA and Tukey's honestly significant difference tests were applied using IBM SPSS Statistics (version 24.0; IBM Corp., Armonk, NY, USA), followed by regression analysis to identify the best-fit model when significant difference was found, and orthogonal polynomial contrast (linear, quadratic, and cubic) was conducted to determine the most suitable model between all dependent variables and the inclusion levels of JMM.

Table 1. Ingredient and chemical composition of the experimental diets (%, DM basis)

| | Experimental Diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | Con | CJ0 | CJ10 | CJ20 | CJ30 | CJ40 | CJ50 |
| *Ingredients (% DM)* | | | | | | | |
| Fish meal | 60.0 | 30.0 | 24.0 | 18.0 | 12.0 | 6.0 | |
| Chicken by-product meal | | 32.2 | 32.2 | 32.2 | 32.2 | 32.2 | 32.2 |
| Jack mackerel meal | | | 6.0 | 12.0 | 18.0 | 24.0 | 30.0 |
| Fermented soybean meal | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| Wheat flour | 10.1 | 9.3 | 9.4 | 9.5 | 9.6 | 9.7 | 9.8 |
| Fish oil | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 |
| Soybean oil | 3.2 | 1.8 | 1.7 | 1.6 | 1.5 | 1.4 | 1.3 |
| Vitamin premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Choline | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| *Nutrients (% DM)* | | | | | | | |
| Dry matter | 95.4 | 95.4 | 95.2 | 95.2 | 95.4 | 95.2 | 95.1 |
| Crude protein | 55.5 | 55.3 | 55.4 | 55.4 | 55.4 | 55.5 | 55.2 |
| Crude lipid | 12.3 | 12.0 | 12.1 | 12.1 | 12.1 | 12.1 | 12.1 |
| Ash | 11.7 | 11.6 | 11.5 | 11.4 | 11.4 | 11.4 | 11.3 |

*(Continued on next page)*

**Results**

At the end of the 8-week feeding trial, weight gain and specific growth rate (SGR) of fish fed the CJ50 were significantly ($P < 0.005$ for both) higher than those of fish fed the CJ0, CJ10, and CJ20 diets. Feed consumption of fish fed the CJ50 diet was significantly ($P < 0.001$) higher than that of fish fed the CJ0 and CJ10 diets. However, no significant ($P > 0.05$) difference in feed utilization was found among dietary treatments. In orthogonal polynomial contrast, weight gain, SGR, and feed consumption of olive flounder were revealed linear ($P = 0.0001$ for all) relationships with inclusion levels of JMM in diets substituting 50% of FM with CBM. Linear relationships were determined to be the most suitable models between inclusion levels of JMM in diets substituting 50% of FM with CBM and weight gain ($Y = 0.073619X + 34.9873$, $P < 0.0001$, $R^2 = 0.7319$), SGR ($Y = 0.000025X + 0.0243$, $P < 0.0001$, $R^2 = 0.6735$), and FC ($Y = 0.083305X + 33.8201$, $P < 0.0001$, $R^2 = 0.6573$) in regression analysis. Economic profit index (EPI) of the CJ50 diet was significantly ($P < 0.01$) higher than that of the Con, CJ0, and CJ10 diets.

**Conclusion**

Inclusion of 50% of JMM in the low-FM diet replacing 50% FM with CBM is the most recommended treatment based on the growth performance and feed availability of olive flounder as well as the economic return (EPI) to farmers.

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# A PORTABLE AND COST-EFFECTIVE STEREO VISION SYSTEM FOR AUTOMATED FISH WEIGHT ESTIMATION IN OFFSHORE AQUACULTURES USING DEEP LEARNING

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## Introduction

Automation technologies are increasingly influencing aquaculture by enhancing fish monitoring, biomass estimation, and feeding management. Traditional manual sampling methods remain labor-intensive, invasive, and subject to bias, underscoring the need for non-intrusive and scalable alternatives (Fernandes et al., 2020). Stereo vision systems combined with deep learning (DL) provide potential solutions; however, existing applications are often expensive, have depth limitations, or are complex to implement. In this study, we present a compact, portable, and cost-effective stereo vision system integrated with a DL model for automated fish weight estimation, developed for offshore aquaculture environments.

## Materials and methods

The proposed system consists of a Luxonis OAK-D PoE stereo camera and a PoE-48V portable power supply, both housed in a waterproof enclosure (Figure 1, left panel). The camera captures synchronized RGB and depth images at 20 frames per second (FPS) and operates within a depth sensing range of 0.7 to 12 meters. The system was deployed in a commercial offshore aquaculture cage managed by Kimagro Fishfarming Ltd. (Figure 1, middle panel) stocked with Mediterranean gilthead seabream (Sparus aurata), following an underwater calibration procedure to optimize the camera parameters. During the field study, the system was suspended 2 meters below the sea surface and recorded continuously for 30 minutes, resulting in 291 RGB-depth image pairs. Simultaneously, 220 fish were manually weighed (mean weight=122.91 g, standard error of the mean (SEM)=1.69 g) to provide ground truth (GT) data. The weighed fish were isolated with a splitting net in the cage to ensure accurate correspondence between estimated and measured weights. Three anatomical keypoints—the snout tip, body midpoint, and middle caudal rays—were detected on the fish body in RGB images using the DL model (YOLOv11n-Pose) and subsequently aligned with corresponding depth data to enable 3D reconstruction of the fish body. Fish length was computed as the Euclidean distance between the three identified keypoints (Figure 1, right panel) and converted to weight using a species-specific empirical length–weight relationship: $W=0.0123L^{3.0284}$ (Mehanna, 2007). An overview of the proposed methodological framework is presented in Figure 2.

## Results and discussion

The system achieved a mean estimated weight of 124.29 g (SEM=6.00 g) after the removal of outliers using the interquartile range (IQR) method, resulting in an absolute error (AE) of 1.39 g and a percentage error (PE) of 1.12% compared to the ground truth (GT) mean weight. Further analysis indicated that training the DL model on ~250 annotated fish instances was sufficient for accurate keypoint localization, while stable weight estimations could be obtained using 60 inference images, corresponding to approximately 9 minutes of camera recording time. Results also demonstrated that including the fish body midpoint as a third keypoint in length and weight estimation improved the system's accuracy and precision by better accounting for body curvature. Outlier analysis further revealed that deviations in weight estimations were primarily associated with artifacts in the depth maps rather than inaccuracies in the DL-based keypoint detections or morphological abnormalities on fish body, highlighting the importance of reliable depth acquisition. The system's compact dimensions (17.8 cm (L) × 10.2 cm (W) × 25.4 cm (H)), low production cost (~€620), minimal operational requirements (9 minutes), and straightforward installation further support its viable application for automated fish biomass monitoring in offshore aquacultures.

## Conclusion

This study demonstrates that integrating a portable stereo vision system with DL enables accurate, efficient, and non-invasive fish weight estimation in offshore aquaculture settings. By reducing time, operational costs, and labor demands, the proposed system represents a robust, accurate, and scalable solution for advancing precision aquaculture practices.

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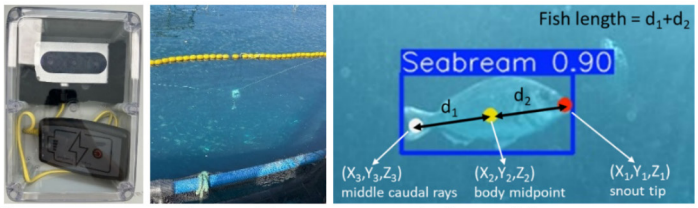


Figure 1: Stereo camera and portable power supply housed in a waterproof enclosure (left), the proposed system operating underwater in the offshore aquaculture cage (middle), and a visual representation of the fish length estimation process (right).

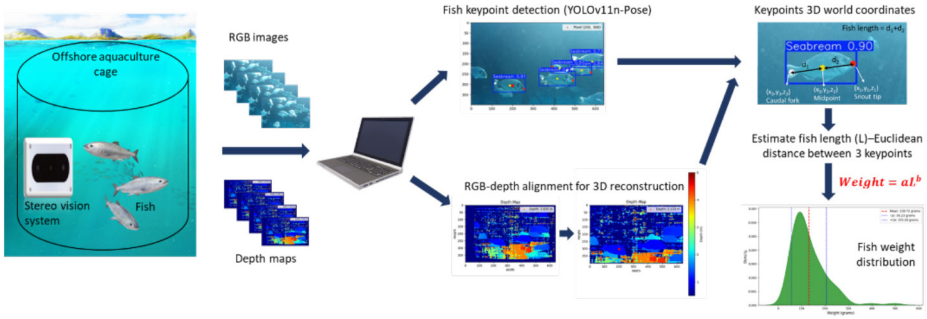


Figure 2: Overview of the methodological framework for automated fish weight estimation using the proposed DL-based stereo vision system.

**Acknowledgments**

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# IMPROVING SEA FARM SUSTAINABILTY AND EFFICIENCY THROUGH AI TECHNOLOGY AND ENVIRONMENTAL OXYGEN PREDICTION

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## Introduction

Aquaculture is one of the main levers for feeding tomorrow's world, but this activity is affected by the global warming and the increase in ocean temperatures, which strongly affect the oxygen level in the water, a key parameter for the fish health and fish appetite -regulated by both temperature and oxygen level-. Oxygen fluctuation will lead to a daily change in fish appetite for Atlantic salmon Salmo *sala***r** *(Remen 2016),* called DFI – *Daily Feed Intake-* and will affect the operations in the sea, mainly on cage feeding management.

In this context, management support through live environmental data is no longer sufficient, and the predictive need becomes essential to adjust daily decisions, in particular feeding actions, which concentrate the environmental impact of the activity, through its production -origin of raw materials- and its use -can be polluting if poorly managed and wasted.

BiOceanOr's predictive tools, based on AI and machine learning from local and external data collection, which help the aquaculture industry by reducing the environmental footprint of activities while maintaining high performance, is one of the solutions to tomorrow's challenges.

## Results

Based on temperature and oxygen prediction (Figure 1), we can identify the ideal and less opportune windows times for feeding during the next working days, anticipating the best appetite window for fish.
By analyzing farmer's state of the art, we can estimate how much feed is given into suboptimal period (Figure 2) and asses the margin of improvement. The Figure 2 below illustrates a pre-deployment analysis in Chile, where 21.2% of the feed is given outside the optimal periods, resulting in feed waste, inefficient productivity, economic loss and unnecessary environmental impact.

By adjusting the feeding time based on the prediction, it becomes possible to move from the red-orange suboptimal zone -21,2% feeding- (Figure 2) to the optimal green zone.

This new feeding management process led to (i) a reduction in the amount of feed not consumed, and improvement in the eFCR of production, (ii) an improvement in the growth performance SGR, by optimising the amount of feed assimilation.

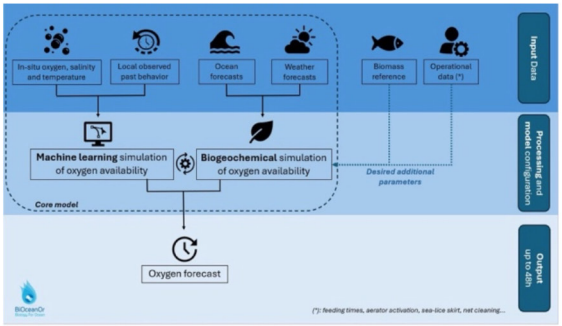


**Figure 1**: AI Technology use -Machine Learning and Biogeochimical model- to enable environmental oxygen forecast in the sea cage production.

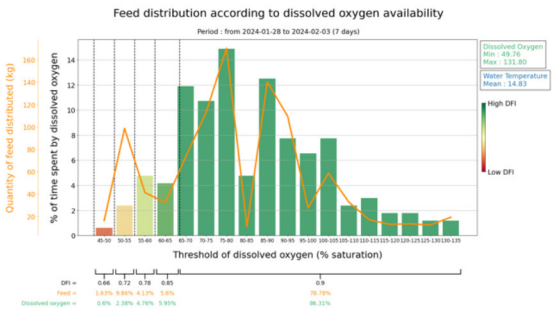


**Figure 2**: : Feeding analysis done before the use of digital tools highlighting the suboptimal feeding done when fish appetite DFI -Salmo *salar* -is low

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**Conclusion**

As the cost of feed to the farmer represents up to 70% of the annual expenditure in the salmon industry and in the carnivorous species in general, this marine oxygen modelling in the sea has a specific value proposition to assist the farmers in their day to day operations, helping them to better manage the feeding process. It is a good example of how technical innovation can lead to both (i) a positive environmental impact through less food lost to wildlife, and (ii) an economic gain for the activity through eFCR & SGR improvement.

**Discussion and perspective**

The tool has been developed through two main innovations, (i) oxygen prediction capacity (Figure 1) and the (ii) specific knowledge of the Atlantic salmon -Salmo *salar*- metabolism. The application to other species is a new challenge, as the effects of global warming are strongly observed in the Mediterranean Sea, where the temperature each summer is approaching the maximum tolerance threshold for commercial fish such as sea bass -Dicentrarchus *labrax*- and sea bream -Sparus *aurata*-, and where the improvement could be much higher than what we use to observe with the Atlantic salmon.

# FADING OF MELANIN SKIN SPOTS: A POTENTIAL FOR NON-INVASIVE AND AUTOMATED MONITORING OF STRESS AND WELFARE IN ATLANTIC SALMON *SALMO SALAR* USING COMPUTER VISION

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## Introduction

Intensive salmon farming is challenged by unsustainable mortality rates, which necessitate the need for new welfare indicators aiming at reducing these losses. Current welfare indicators in the industry are either invasive or yield a signal only after a problem has developed (e.g. external indicators such as emaciation, wounds, scale loss, etc). Previously, it has been shown that heritable variation in stress responsiveness is reflected in melanin pigment patterns in several vertebrate species, including salmonid fish (Kittilsen et al., 2009). In the case of Atlantic salmon and rainbow trout, high post-stress cortisol levels are specifically associated with a reduced incidence of melanin – based markings (i.e., black skin spots). In the current study, we have investigated the possibility of using these spots as a non-invasive indicator of welfare in salmon based on our hypothesis that fading (or bleaching) of these spots occur in stressed salmon.

## Methods and Methodology

A dataset of 130 salmon was collected by photographing each fish before and after a 17-hour standardized confinement stress, during which they were kept in a tank with reduced water levels until their dorsal fins were completely submerged. All images were standardized using image-preprocessing technique based on the darkest and brightest pixels in each image. These processed images were used to train computer vision models to identify the operculum region and detect spots within it. Spot localization data from the model was then used to extract grayscale values and spot areas, which were analyzed to assess changes in response to stress.

## Results and Discussion

The grayscale values and areas of 676 uniquely identified spots were compared before and after stress. The grayscale values were clustered and plotted on a grayscale. We observed significant bleaching, as well as a reduction in the areas of the post-stress spots (P-value < 0.0001, two-way ANOVA). The spots were also analyzed sidewise, but no significant effect was observed on bleaching or the changes in spot areas.

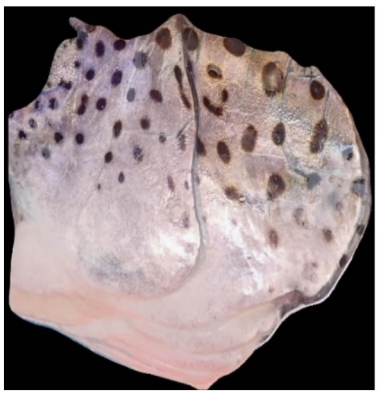

Fig 1: Left side operculum region of interest

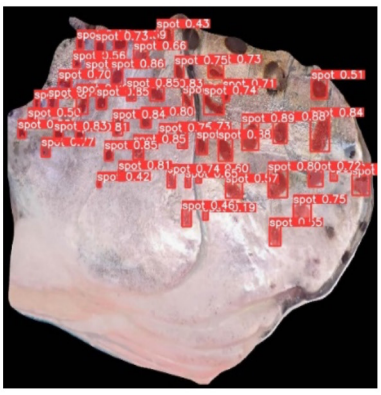

Fig 2: Detected Spots in operculum ROI

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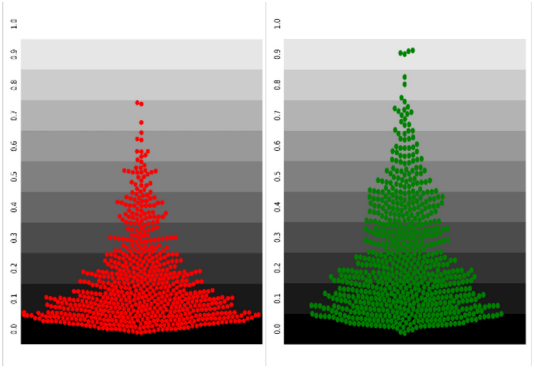

Fig 3: The red cluster (mean = 0.1934, median = 0.144) represents pre-stress grayscale values, while the green cluster (mean = 0.275, median = 0.22) shows post-stress values.

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# ANTIBACTERIAL MICROENCAPSULATED PEPTIDE AS AN IMMUNOSTIMULANT ADDITIVE FOR SALMON

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This work addresses a significant challenge in the Chilean salmon farming industry, the world's second-largest producer, by developing an innovative microencapsulated feed additive containing recombinant antimicrobial peptides derived from the innate immunity of salmonids (CATH-FLA). The aim is to reduce dependence on antibiotics and combat diseases such as *Piscirickettsia salmonis*. Using bioinformatics and *E. coli* fermentation, the chitosan-encapsulated peptides provide gastric protection and targeted intestinal delivery, thereby reducing pathogen-induced cell death while minimizing inflammation. In this work, we scale up the production to 20L fermentation and evaluate the stability and biological activity of chitosan-microencapsulated CATH-FLA peptide under different storage conditions over 120 days. Results demonstrated that the microencapsulated peptide remained stable at 10°C and 20°C for 120 days, with minimal release at pH 2, and controlled release at pH 8. Scanning electron microscopy revealed pseudo-spherical microparticles with temperature-dependent size reduction over time, with the smallest particles observed at 37°C. Biological activity evaluated in SHK-1 salmon kidney cells treated with the pH 8-released peptide showed significant upregulation of pro-inflammatory markers IL-1β (1.5–2.5-fold) and IL-8 (2.0–2.7-fold), suggesting that CATH-FLA maintains its immunostimulatory properties. The optimal storage temperature was determined to be 4°C, balancing stability and particle integrity. This study supports the potential of chitosan-microencapsulated CATH-FLA as a stable and bioactive delivery system for aquaculture applications. This sustainable solution could cut antibiotic use by 10–20%, lower production costs, and improve market competitiveness, aligning with global demand for eco-friendly aquaculture.

# INCORPORATING PALM KERNEL MEAL, COWPEA HUSK AND SOYBEAN HUSK AS PROTEIN SOURCES IN CATFISH DIETS: EFFECTS ON GROWTH, HEMATOLOGY AND INTESTINAL HISTOLOGY

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## Introduction

Feed is necessary in aquaculture, just like in animal husbandry, to guarantee good production, particularly in intensive culture systems that demand a lot of input (Boyd, 2020). _Since feed makes up over 50% of the entire production expenses in the aquaculture business, feed is a crucial component (Mzengereza et al., 2014). The costliest feed ingredient in aquafeeds is the source of protein (Lim et al., 2023). Fishmeal is considered the gold standard protein due to its balanced amino acid content, which is perfect for the healthy growth and development of fish (Perez-Velazquez et al., 2019). Fish by-products and wild-caught fish are used to make fish-meal, an industrial product (Wan, 2015)._Fishmeal is a major source of aquafeed worldwide. However, the rising demand for fish and fish products has caused its price to continuously rise, greatly affecting the market price (Jannathulla et al., 2019). To cut feed costs, several plant-based proteins are now utilized in place of fish-meal, either totally or partially (Jannathulla et al., 2019). The main objective of using plant-based proteins is to offer fish-meal as a more affordable substitute. Finding a less expensive option isn't the only factor, though (Gutasi, 2021). Moreover, the production of these plant-based proteins needs to be sustainable without affecting the health and growth of fish (Magbanua and Ragaza, 2024). Consequently, the aim of this study was to evaluate the effects of soybean husk, cowpea husk, and palm kernel meal as protein sources on growth performance, feed utilization, intestinal histology, and health of catfish using full blood count and liver function test as indicators.

## Materials and Methods

### *Formulation of the experimental diets and feeding*

Table 1 shows the experimental diets used for this study. In formulating the diets, substantial amount of fish meal was progressively replaced with soybean husk (SBH), cowpea husk (CPH) and palm kernel meal (PKM) until equal amounts of protein (approximately 35%) were obtained. Following procedures previously described by Ayisi et al. (2017), the formulated diets were prepared. *C. gariepinus* were fed to apparent satiation, three times daily at 8:00 am, 12 noon and 4:00 pm for ten weeks.

### *2.2. Experimental conditions and design*

The experiment was carried out in concrete tanks with dimensions of 1.5 m x 1.5m x 1.2 m (length, breadth and depth). Five hundred healthy *C. gariepinus* with initial weight of approximately 2.5 g were purchased from a commercial farm and transported to the rearing facility. Prior to the onset of the trial, fish were acclimatized to the rearing conditions for 2 weeks and fed on commercial diet with protein content of 32%. At the onset of the trial, the fish were distributed into four groups with duplicates and fed their respective diets. Each tank was stocked with fifty fish.

### *2.3 Measurements of the biological indices*

Feed utilization and growth performance were measured following formulas previously used by Mutalib et al. (2023).

### *2.4 Blood biochemical analysis*

After 10 weeks of feeding trial, blood from experimental fish were sampled and blood biochemical analysis performed as previously described by Abarike et al. (2018).

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### *2.5 Proximate composition*

Samples from whole body fish and experimental diets were sent to the laboratory for protein, lipid, moisture, and ash analyses. The AOAC (2003) standard methods, as reported by Ayisi et al. (2021), were employed to analyze the aforementioned parameters.

### *2.6 Histological examination*

Six fish per group had sections of their intestines removed, which were then quickly preserved in a Bouin solution for histological examinations. Samples of tissue were promptly preserved in a Bouin solution. After that, fixed samples were subjected to a series of alcohol solutions with increasing grades (70–100 percent) to dehydrate them. Following the dehydration procedure, tissues underwent xylene deparaffinization, paraffin embedding, 5 µm sectioning, and Hematoxylin and Eosin (H&E) staining (Ismail, et al. 2020). A Leica light microscope was used to examine the slides, and an eyepiece camera was used to capture the images. ImageJ version 1.54 was used to determine the target cells in each section.s

### *2.7 Statistical analysis*

The gathered data was statistically analyzed using Graph Pad Prism (V.5.03). The data is presented in tables using the standard error of the mean (SEM), which is represented as the mean ±. To compare treatment means, One-way Analysis of Variance and Tukey's multiple tests were applied to all the data. Differences are considered significant for all data at the 0.05 probability level ($P<0.05$). Pearson's correlation was conducted to ascertain the relationship between selected parameters. Results of correlation analysis is presented in figures.

## Results

The results indicated that inclusion of these alternative protein sources had significant effects on growth performance and feed utilization compared to FM diet. The weight gain recorded in this study was as follows: SBH (119.5±68.02) > CPH (113.2±53.14) > FM (104.3±56.82) > PKM (86.73±31.51). Feed conversion ratio ranged from 1.25±0.57 (SBH) to 1.52±0.56 (PKM). *C. gariepinus* fed the diet PKM had the lowest protein efficiency ratio (2.09±0.76), followed by those fed the FM (2.57±1.40) and the CPH diet (2.74±1.28). To a larger extent, the dietary protein sources significantly influenced serum hematology parameters. *C. gariepinus* fed the FM diet had the highest white blood cells count (133.0±2.51), which was significantly higher than all other groups ($p < 0.0001$). The range of red blood cell values observed in this study was 2.08±0.13 to 2.62±0.13. Histological examination indicated modifications in intestinal morphology, suggesting possible metabolic adjustments to the experimental diets. Fish fed the FM had the highest villus height (441.2±22.6), followed by SBH (398.3±7.51), PKM (279.2±15.65), and CPH (142.3±10.84). Villus width and muscular thickness also followed this pattern, with fish fed the FM diet having the largest villus width (153.7±9.06), significantly greater than all other groups ($p<0.0001$). Overall, incorporating CPH, and SBH into catfish diets appears to be a viable replacement for conventional fishmeal-based diets; however, further studies are required to determine optimal inclusion levels for achieving maximum growth and well-being.

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# NEW TECHNOLOGY FOR MAPPING OF DARK SPOTS IN SALMON FILLETS

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Dark spots in salmon fillets represent a significant and costly quality issue for the aquaculture industry; distinct and large spots have been reported to cause an economical loss of 24 to 44%. Previous surveys have shown that the occurrence of dark spots in Norwegian salmon farming averages around 15-20%, with considerable variation between fish groups.

The current project aims to continue the survey by first developing new technology for easy and efficient registration of dark spots on the fillet line. In addition to presenting preliminary data from the survey, the presentation will showcase the technology developed in the project: 1) a camera for automatic detection of dark spots through image analysis; 2) a touchscreen-based solution for manual registration. Data from the registrations are collected in a database, where it is anonymized and used for mapping. Users can also visualize and download their own registrations.

The survey has the potential to form an important data basis for the search for causal relationships. Better knowledge of this complex quality deviation could help reduce its extent, thereby increasing the yield in salmon aquaculture, which is considered important in the work towards sustainable aquaculture.

# ASSESSING GROWTH PROMOTION BY AQUACULTURE FEED SUPPLEMENTS USING *Danio rerio* AS A MODEL ORGANISM

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The zebrafish (*Danio rerio*) has become an established model organism across multiple research fields. In terms of aquaculture, some international groups have recently used it as a model species for feeding assays due to its rapid development, small size, suitability for high-throughput experimentation and its deep genetic knowledge. Although species-specific differences exist, zebrafish offers a practical platform for the early-stage evaluation of aquafeed supplements, providing valuable insights into the nutritional regulation of growth prior to conducting large-scale trials in commercial finfish species.

Recent studies have demonstrated the utility of zebrafish in assessing dietary effects on growth performance. The present work is part of a broader research initiative aimed at improving sustainability and productivity in aquaculture through the development of functional feeds, supported by two interrelated experimental grants. These projects explore feed supplementation with compounds of microbial and algal origin, such as marine polysaccharides or extracts and bacterial extracellular polymers (ECPs), selected for their potential to enhance fish growth and resilience against pathogens of economic relevance.

In this communication, we present results from our research group evaluating the effects of selected feed supplements on zebrafish growth during the presexual maturity stage and under controlled water temperature, salinity, light, and dissolved oxygen (DO) conditions. After a generalized live food regime and the palatability studies, three different functional feeds were daily administrated “ad libitum” to a constant population of one-month fishes under a constant feeding regime to reduce potential compensatory growth. Non-supplemented or commercial feeds were also administrated to negative or positive control groups. During this procedure, stocking density, natural size heterogeneity, food particle size and ration level were also controlled. Viability, initial and final body lengths and weights were measured and plotted to analyse either global or separately subpopulations of low-, medium-, and heavy-weights. Parameters regarding coefficients of variations in weight, body mass index, body condition scoring, and the nutritional properties of food were also considered.

Nonetheless, careful consideration must be given when extrapolating zebrafish data to commercially relevant species, due to physiological and ecological differences. These limitations emphasize the need for greater standardization in zebrafish research and a cautious interpretation of findings when applied to industrial aquaculture.

# DEMAND COVERAGE IS NOT ENOUGH: THE INFLUENCE OF VARYING LYS TO MET + CYS RATIOS ON FEED INTAKE IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

Fishmeal is still one of the main protein sources in fish feed, but its increasing demand can only be covered by sustainable aquaculture that uses alternative protein sources (FAO, 2022; Hua et al., 2019; Sarker et al., 2020). Therefore, in this project, raw materials that were suitable for organic production were used. Although plant-based protein sources have been tested as substitutes for fishmeal, these often have negative effects on digestion, taste, and growth due to a suboptimal amino acid composition (de Francesco et al., 2004; Francis et al., 2001; Lazzarotto et al., 2018; Kaiser, 2021; Nagel et al., 2012; Slawski, 2012). There are also indications that an amino acid imbalance could reduce feed intake (Bak et al., 2012; Comesaña et al., 2017; Comesaña et al., 2020). The purpose of this study was to test raw materials as fishmeal substitutes to investigate the influence of varying Lys to Met+ Cys ratios above demand, because it is assumed that these higher ratios can positively affect feed intake and growth.

## Material and Methods

Six different diets were formulated, which varied in terms of raw material and amino acid composition in terms of Lys to Met+ Cys ratios. The control diet included fishmeal and soy protein as the main protein sources. In five experimental diets, the fishmeal and soy protein were completely replaced by different combinations of pea protein, poultry meal, soldier fly larvae meal, and feather meal, resulting in different dietary amino acid compositions in terms of Lys to Met+ Cys ratios (Table 1). All diets were isonitrogenous and isoenergetic at the digestible level and were tested in triplicates. Rainbow trout with an initial weight of ~26g were held in a recirculation aquaculture system and fed daily to apparent satiation for 52 days.

## Results and Discussion

The group fed the diet with a lysine to methionine+ cysteine ratio of 1:0.40 showed a significantly higher DFI (3.11±0.06) than the overall mean. Fish fed the diet with a ratio of 1L:0.49M+C showed a significantly lower FCR (1.05±0.03). Both diets showed a higher SGR (2.86±0.03). Both treatments contained the highest levels of pea protein (25.1%; 17.4%), which, according to Gatlin III et al. (2007), is suitable as a moderate fishmeal substitute as a high proportion of lysine and methionine is provided. The amino acid composition might affect the higher feed intake of the diet 1L:0.40M+C. This diet contained the highest levels of isoleucine, leucine and arginine. According to Yamashita, Yamada & Hara (2005), isoleucine and leucine have a positive effect on feed intake in rainbow trout. However, Comesaña et al. (2020) showed a negative effect on the provision of leucine on feed intake. Arginine is an effective taste stimulant in fish, but its influence is negligible due to the husbandry conditions prevailing in this experiment, as their receptors are active only at a pH of 8.5 (Marui et al., 1983). The SGR and FCR values of the diet 1L:0.49M+C could also be attributed to the amino acid composition, with a methionine and cysteine ratio of 1:1, which may benefit starch and fat digestion (Nordrum et al.,1999). Some studies showed that cysteine might be a positive stimulant for locomotory activity in rainbow trout and feed intake (Yamashita, Yamada & Hara, 2005; Kasumyan & Doving, 2003).

*Table 1: Dietary amino acid contents of Lysine and Methionine+ Cysteine in g per 100g dry matter of the test diets (Treatment=Name). Tested ratios of the amino acids and the respective amino acid requirement for rainbow trout (Rodehutscord et al. 1997, Hardy & Kaushik 2021).*

| Treatment | Lys (dietary) | Met+ Cys (dietary) | Ratio Lys: Met+ Cys | Demand Lys | Demand Met+ Cys |
|---|---|---|---|---|---|
| $1_L{:}0.40_{M+C}$ | 3.0 | 1.20 | 1:0.40 | 2,77 | 1,2 |
| $1_L{:}0.43_{M+C}$ | 2.93 | 1.27 | 1:0.43 | | |
| $1_L{:}0.49_{M+C}$ | 2.86 | 1.40 | 1:0.49 | | |
| $1_L{:}0.56_{M+C}$ | 2.93 | 1.63 | 1:0.56 | | |
| $1_L{:}0.69_{M+C}$ | 2.77 | 1.92 | 1:0.69 | | |

*(Continued on next page)*

**Conclusion**

The results show that organically produced raw materials in fish nutrition can offer sustainable alternatives to conventionally produced feeds. It could also be demonstrated that feed formulations with different ratios of lysine to methionine+ cysteine significantly influence feed intake, feed conversion and specific growth rate in fish. In addition, the ratio of methionine to cysteine appears to influence nutrient digestion, which could improve growth performance. Follow-up experiments examine more closely the optimal balance of these amino acids and the potential of ecological resources to maximise feed efficiency and growth in aquaculture.

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# PROTEIN-SPARING EFFECTS OF A MODIFIED HIGH FAT DIET IN PACIFIC WHITELEG SHRIMP *Litopenaeus vannamei*

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## Introduction

Aquaculture significantly contributes to global seafood production (FAO, 2022) and *Litopenaeus vannamei* is one of the key aquaculture species due to its adaptability and disease resistance (Danya Babu Ravuru, 2015). However, high feed costs necessitate alternative strategies like protein-sparing, which involves substituting dietary protein with lipids (Wang et al., 2015). This study evaluated the protein-sparing effects of modified diets on the growth performance and metabolic effects on *L. vannamei*. Diet modification was made by adding the following compounds: *L*-carnitine, choline chloride, glycerol monolaurate, and lysophospholipids.

## Materials and Methods

All diet feeds were produced in-house at VDS, N.V. in Deerlijk, Belgium. The study consisted of two feeding trials conducted over 57 days.

The initial average weight of the shrimp for the first feeding trial was 11.19±0.06 grams. For the second feeding trial, the initial average weight of the shrimp was 0.86±0.00 gram. Shrimps were cultured in a recirculating aquaculture system. Water quality parameters were monitored and controlled to ensure optimal culture conditions. At the end of the feeding trials, growth performance metrics and metabolic effects were determined.

## Results

For the first feeding trial (*Table 1*), the MPOS treatment had significantly higher body weight than the NEG treatment. Other parameters did not show significant differences among the treatments. It is also noteworthy that the MPOS showed better growth performance tendencies, although not significant.

In terms of whole body protein (*Figure 1*, left), NEG treatment had an expectedly significant lower body protein content (%) compared to other treatments and the initial body protein, indicating low protein assimilation from a low protein diet. This effect was mitigated by the modification of the NEG diet (MNEG). For the whole body fat content (*Figure 1*, right), there was an expected significant increase of whole body fat of treatments NEG and MNEG. It appears that diet modification did not decrease the body fat of the shrimps.

For the second feeding trial, the results confirmed the previous feeding trial results on POS and MPOS (*Table 2*). Additionally, the Protein Efficiency Ratio (PER) of MCON was significantly higher than CON treatment.

## Conclusion

The diet modification showed best results in a high-protein, low-fat diet of shrimp throughout its growing stage (from 0.8 gram to 27 grams). It also improved some growth parameters and metabolic effects in the low protein, high-fat diet, indicating an amelioration of its negative effects. Additionally, the diet modification demonstrated a protein-sparing effect in the high-fat diet, as confirmed by the whole body protein analysis of the shrimp, although it did not reduce the total body fat content.

*(Continued on next page)*

| First Feeding Trial | |
|---|---|
| **Treatment (%Protein/%Fat)** | **Designation** |
| POS (34/7) | Positive control |
| NEG (31/10) | Negative control |
| MPOS (34/7) | Modified positive control |
| MNEG (31/10) | Modified negative control |

| Second Feeding Trial | |
|---|---|
| **Treatment (%Protein/%Fat)** | **Designation** |
| CON (42/7) | Control |
| MCON (42/7) | Modified control |

Table 1. Growth performance parameters of *L. vannamei* in the first feeding trial. The * superscript indicates significant mean differences at $p<0.05$.

| **Growth Parameters** | **Treatment** | | | |
|---|---|---|---|---|
| | **POS** | **NEG** | **MPOS** | **MNEG** |
| Feed Conversion Ratio (FCR) | 1.74±0.04 | 1.92±0.12 | 1.60±0.15 | 1.94±0.17 |
| Ave. Body Weight (ABW), *g* | 26.78±0.69 | 26.04±0.26* | 27.29±0.88* | 26.47±1.32 |
| Ave. Body Length (ABL), *cm* | 14.66±0.11 | 14.92±0.19* | 14.71±0.06* | 14.88±0.31 |
| Ave. Weekly Gain (AWG), *g* | 2.11±0.13 | 2.00±0.04 | 2.17±0.12 | 2.04±0.18 |
| Specific Growth Rate (SGR), *%* | 1.70±0.06 | 1.63±0.02 | 1.72±0.06 | 1.64±0.09 |
| Survival Rate (SR), *%* | 95.00±2.83 | 96.50±4.95 | 98.50±2.12 | 97.00±0.00 |
| Protein Efficiency Ratio (PER), *%* | 6.13±0.38 | 5.91±0.14 | 6.23±0.34 | 5.93±0.52 |

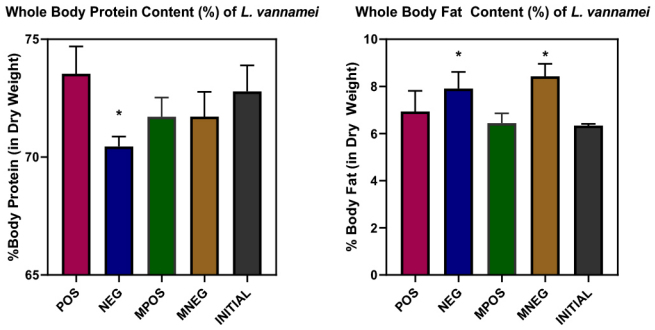


Figure 1. *Left*: Whole body protein content (%) of *L. vannamei*. *Right*: Whole body fat content (%) of L. vannamei. * indicates significant mean differences at $p<0.05$.

Table 2. Growth performance parameters of *L. vannamei* in the second feeding trial. The * superscript indicates significant mean differences at $p<0.05$.

| **Growth Parameters** | **Treatment** | |
|---|---|---|
| | **CON** | **MCON** |
| Feed Conversion Ratio (FCR) | 1.46±0.07 | 1.35±0.08 |
| Ave. Body Weight (ABW), *g* | 12.50±0.24 | 12.61±0.11 |
| Ave. Body Length (ABL), *cm* | 11.37±0.52 | 11.92±0.18 |
| Ave. Weekly Gain (AWG), *g* | 1.96±0.05 | 1.94±0.06 |
| Specific Growth Rate (SGR), *%* | 6.41±0.05 | 6.39±0.07 |
| Survival Rate (SR), *%* | 92.50±4.80 | 93.75±2.63 |
| Protein Efficiency Ratio (PER), *%* | 2.21±0.06* | 2.37±0.08* |

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# INTERPLAY BETWEEN UV, OZONE AND THE PARASITE *Spironucleus salmonicida* IN ATLANTIC SALMON RAS

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## Introduction

Recirculating aquaculture systems (RAS) are increasingly being adopted for land-based Atlantic salmon farming in Norway. While RAS provides relatively higher biosecurity compared to flow-through systems, disease outbreaks can still occur. One such disease is spironucleosis, which may be introduced into the system via the water intake. In Northern Norway, outbreaks of spironucleosis in farmed salmon remain a persistent problem, leading to mass mortality, compromised welfare and significant economic losses.

The diplomonad parasite *Spironucleus salmonicida* (Figure 1) is the causative agent of these systemic infections, which are characterised by internal haemorrhaging, splenomegaly, and granulomatous lesions in the liver and spleen. Environmental conditions significantly influence the severity and distribution of the disease. Currently, no known treatment for spironucleosis exists, making prevention the primary means of control. Anecdotal reports from the industry suggest that the parasite may be sensitive to UV disinfection.

This study investigated the use of UV and ozone as water disinfection methods within the RAS loop to prevent spironucleosis outbreaks following a biosecurity breach in Atlantic salmon RAS.

## Materials and methods

*In situ* and *in vivo* trials were conducted to evaluate the effects of disinfection on the viability and potential proliferation of *Spironucleus salmonicida* in RAS.

In the *in situ* trial, the survival of *S. salmonicida* flagellates following UV treatment was assessed under controlled laboratory conditions. The flagellates were exposed to two types of UV lamps: low-pressure (dose range: 10–200 mJ/cm²) and medium-pressure (dose range: 10–75 mJ/cm²). Parasite survival was evaluated immediately after UV exposure and again at 24 and 48 hours post-treatment.

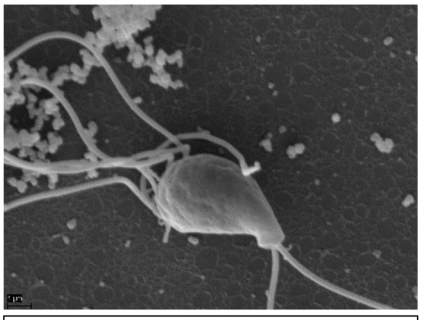

Figure 1. *S. salmonicida* under SEM.

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In the *in vivo* trial, brackish water-adapted Atlantic salmon smolts were reared in nine individual RAS units. Three units received UV disinfection in the RAS loop at a continuous dose of 100 mJ/cm². Another three units were disinfected using ozone, targeting an oxidation-reduction potential (ORP) of 320–350 mV. The remaining three units, which received no RAS loop disinfection, served as controls.

The parasite (approximately $10^5$ cells/mL) was introduced via the make-up water over three consecutive days at a concentration of 1% (v/v) of the total daily water volume (approximately 20 L/day). Disease progression was monitored over a four-week period, including assessments of water quality parameters, collection of swab samples for microbial profiling, and tissue sampling for histology and qPCR analysis.

**Results**

No parasites survived treatment with medium-pressure UV lamps at a dose of 50 mJ/cm² or higher at any time point. At lower doses (10 and 25 mJ/cm²), the parasites were not viable over time and were unable to reproduce, despite appearing alive under the microscope at 0 and 24 hours post-treatment. However, they were significantly weakened and all died within 48 hours.

Water quality in the nine experimental RAS units remained within favourable thresholds for Atlantic salmon smolts. UV transmittance was highest in the UV-treated units and lowest in the control group.

All fish tested negative for the presence of the parasite. Molecular markers associated with innate immunity and stress response were differentially regulated in the foregut, hindgut, skin, and gills. Histological indicators of skin and gill health changed significantly over time, particularly in the control group, although inter-treatment differences were minimal.

RAS loop disinfection influenced the microbial communities in the system, including those on tank walls and biomedia. Microbial composition evolved over time in all treatment groups. Certain members of Alphaproteobacteria were enriched in the ozone-treated group but not in the UV-treated group, relative to the control.

**Conclusion**

Medium-pressure UV treatment at 50 mJ/cm² effectively inactivated *Spironucleus salmonicida*. The *in vivo* biosecurity breach of the parasite in RAS did not cause infection, and water quality remained within optimal ranges across all RAS units. Disinfection methods influenced microbial community composition, particularly with ozone, which enriched certain Alphaproteobacteria.

**Acknowledgement**

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# ATLANTIC SALMON BROODSTOCK ENVIRONMENT AND ITS EFFECTS ON OFFSPRING IMMUNE AND STRESS RESPONSES

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## Introduction

Genetic variation is key in determining traits important for Atlantic salmon aquaculture, and selective breeding has been widely used to improve performance. However, environmental conditions such as nutrition, handling, disease exposure, temperature, and oxygen availability also significantly affect the development and health of fish. Increasing attention is being given to how the broodstock (G0) environment may influence the performance of their offspring (G1), including immune function and stress resilience. In Norwegian aquaculture, different rearing and maturation practices across breeding and multiplier companies may be contributing to variation in offspring health and robustness. Understanding these environmental influences is essential for improving fish welfare and production outcomes. As part of the project, EpiBrood, we explore how variations in broodstock rearing conditions affect immune and stress responses in the offspring of Atlantic salmon.

## Materials and methods

Two major salmon egg producers provided experimental materials for this project. Eggs from Producer 1 originated from broodstock reared in southern and mid-Norway. In contrast, eggs from Producer 2 came from broodstock reared in three different locations: on-land and at-sea facilities in Norway, and an on-land facility in Iceland. Before the first feeding stage, the alevins were subjected to transport stress and a pathogen challenge. When fish from Producer 1 reached approximately 20 grams, they underwent a stress test. Additionally, head kidney leukocytes and hepatocytes were isolated, cultured, and exposed to various model stressors for further analysis.

## Results

Alevins from Producer 1 showed no significant mortality following transport. However, after 24 hours, alevins originating from Mid-Norway had higher survival rates than those from South-Norway. Following exposure to *Yersinia ruckeri*, one Mid-Norway group demonstrated a higher survival rate than the other group. This difference was partially supported by gene expression profiles related to inflammation and antibacterial defence at mucosal sites. Upon exposure to handling and confinement stress, parr from Mid-Norway exhibited a higher cortisol response than parr from South-Norway. At the cellular level, exposure to stressors simulating oxidative stress, hypoxia, and bacterial infection revealed no consistent origin-dependent patterns.

Similarly, alevins from Producer 2 did not exhibit transport-related mortality. However, after 24 hours, alevins derived from Norwegian broodstock had higher survival rates than those from Icelandic broodstock. No clear differences were observed in gene expression profiles between the groups following pathogen exposure. Nonetheless, behavioural differences were noted among the groups.

## Conclusion

The broodstock environment had variable effects on immune and stress responses of the offspring, with some physiological attributes showing origin-dependent differences. These findings suggest that parental rearing conditions can influence offspring robustness and should be considered in broodstock management strategies.

## Acknowledgement

The study was financed by the Norwegian Seafood Research Fund (EpiBrood #901923).

# BIO-LOGGING THE HEART RATE RESPONSES TO EXERCISE TRAINING AND HIGH TEMPERATURES IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

Heatwaves are causing a serious threat to rainbow trout aquaculture (FAO, 2020) and there is urgent need to find solutions how the resilience of fish against heatwaves could be increased. One organ that is influenced by temperature is heart and at high temperatures cardiac arrhythmias are commonly observed (Eliason and Anttila, 2017). Therefore, one way the thermal resilience of aquaculture fish might be increased could be via increasing the cardiac performance of fish. Exercise training has been shown to improve the cardiovascular functions in fish (Palstra and Planas, 2011) and evidence suggests that exercise may even enhance their cardiovascular thermal tolerance (Pettinau et al., 2022). Nevertheless, it is unclear how heart rate changes during the exercise training program and thermal stress challenge. One innovative method to investigate these changes is with bio-logging. Bio-loggers allow continuous collection of heart rate data while the fish are swimming or resting while also continuously monitoring adaptations through time. In this study, bio-loggers were used to assess heart rate variability of rainbow trout during a 4-week exercise training period and thermal stress. The main aim was to get insights how fast the exercise training affects heart rate in fish during swimming and resting to optimize the training duration for aquaculture purposes. Furthermore, the aim was to investigate how training affects the heart rate during a temperature stress.

## Materials and Methods

Rainbow trout (n=115) were acclimated for 1 month to experimental tanks with continuous flow-through freshwater. After acclimation, 40 fish were used for surgical bio-logger implantation. Fish were allowed four weeks to recover from the surgery. The trout were then divided into two groups (control and trained) to triplicate tanks (n=19 fish per experimental tank) including 20 fish with the bio-logger in each group. The loggers recorded the heart rate every 1 hour, for 10 seconds/time at a frequency of 150Hz. Fish were swimming at water velocities of 0.3 body lengths per second (bl/s) for controls and 0.9 bl/s for trained, from 8:30 am - 2:30 pm, 5 days per week for 4 weeks. Weekends were recovery periods as well as rest of the day for trained fish when the velocity was reduced to 0.3 bl/s. After four weeks of training the critical thermal tolerance ($CT_{max}$) of the fish was measured. For this both the trained and untrained fish (n=20/group) were placed in a temperature-controlled tank where temperature was elevated at a rate of 2°C per hour (started from 18°C). Fish were monitored for loss of equilibrium (LOE) at which point fish were determine to have reached $CT_{max}$, bio-loggers recorded the heart rate every 5 minutes during the temperature challenge.

## Results

The heart rate changed significantly during the training period. During the first three weeks the trained fish had significantly elevated heart rate during the exercise periods ($p<0.05$) and even during the resting periods with few exceptional days when the difference did not reach statistical significance. However, by the beginning of week four, the heart rate of the trained fish returned to control levels (no significant differences, $p>0.05$) even for training periods. From the mid-week of week four onwards the resting heart rate of trained fish decreased compared to control group ($p<0.05$).

While the average heart rate of trained fish was higher during the $CT_{max}$ experiment ($P<0.05$) than the one of control fish, there were no significant statistical differences in the CTmax temperature or the temperature where cardiac arrhythmias were observed between groups.

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**Discussion**

The results show that training is effective tool to modify the cardiac function of fish, but it takes more than three weeks to achieve significant functional improvements i.e. efficiency of contraction improving so much that heart rate do not need to be elevated during swimming. However, the training program did not influence the acute critical thermal tolerance of resting fish even though they were able to keep higher heart rate during the challenge. Further studies are needed to see if the training can be optimized so that also in the resting fish the thermal tolerance can be improved as previously shown with maximal heart performance (Pettinau et al. 2022).

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# DEVELOPMENT OF A BIO-SOURCED AQUACULTURE FEED AND BAIT FOR CRUSTACEAN CULTIVATION AND FISHERIES

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Microalgae and insects are becoming a vital part of aquaculture feeds, offering a sustainable and nutritious alternative to traditional ingredients at the basis of feed formulation. They provide essential nutrients like long-chain polyunsaturated fatty acids, amino acids, proteins, and pigments, which are crucial for larval survival and growth in fish and shellfish. They are increasingly being considered as sustainable nutrients sources for aquaculture feeds due to their high protein content, ability to recycle organic waste, and recycling of resources. Another yet untargeted potential use of insects and microalgae is the production of alternative baits for crustacean fisheries. Indeed, conservation challenges are rapidly unfolding in crustacean fisheries relying heavily on declining, resource-consuming forage fish for use as bait. The "bait crisis" is forcing stakeholders to take measure of the situation and to rapidly develop alternative strategies. The most effective, the identification of viable replacements for the fish-based traditional bait, could reduce fishing pressure, stem forage fish depletion and eliminate the negative impacts of fluctuations in bait supply availability, prices and observed unsustainability. The aim of this review is to provide general information of the fisheries, ecology and biology of the American lobster (*Homarus americanus*) and the snow crab (*Chionoecetes opilio*) and summarize the latest research efforts aimed at the identification of suitable alternative baits for their harvesting and/or as feed ingredients for their cultivation. We discuss the use of emergent sources free from aquatic resources fluctuations and regulations. The concept is based on the use of cost-efficient innovative technologies and circular economy transformations of industrial or agriculture side stream by-products (ex: carbon dioxide ($CO_2$) or food wastes) as primary source for sustainable production of low trophic species such as microalgae or insects. This biomass in turn may be used as the main constituent or as an attractant (or a supplement) in the composition of alternative feeds and baits for the crustacean fisheries towards sustainable profitability. Major challenges still reside in the development of a sustainable alternative ingredients market: the emergence of efficient large-scale cultivation technologies and the creation of attractive species-specific olfactive signatures that will promote movement patterns that translate in survival, growth and/or catchability. If carried out and proven cost-effective, the use of emergent sources could significantly contribute to the alleviation of ecosystem collapse due to overexploitation of the pelagic fish populations.

# HIGHER DIETARY EPA & DHA LEVELS IMPROVE COMMERCIAL SALMON FARMING PRODUCTIVITY AND PREDICTABILITY

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## Introduction

It has been widely reported that investing in Omega-3 EPA & DHA rich feeds boosts fish health and consistently produces better quality whole fish and fillets across the value chain. But how do we know? And what's the return on investment in better Omega nutrition for salmon farmers? The objective of this study is to help further close the gap between nutritional science and commercial feeding practice, using so-called 'big data'.

## Methods

This study compared the performance and quality differences of almost half a billion Atlantic salmon on commercial farms in Norway over a 10-year period based on varying dietary levels of EPA & DHA. The study method involved preparing large-scale, complex commercial datasets, or 'big data', and interrogating this with Inverse Probability Weighted Regression Adjustment (IPWRA) analysis to quantify the benefits of increased dietary EPA & DHA levels on salmon populations.

## Results

This study confirmed that higher dietary levels of EPA & DHA lead to significant improvements across a range of production metrics, including an 8% reduction in total mortality, a 13% improvement in economic Feed Conversion Ratio (eFCR) and a 2% increase in superior quality at harvest. Improvements in predictability also increased by up to 50%. Data from processors revealed that above-average levels of EPA & DHA in fish feed consistently delivered better flesh quality, including a 7% melanosis and a 40% blood spots reduction.

## Conclusions

We conclude that these results are extremely important for the salmon industry. It provides valuable insights for strategic, data-driven decision making by importers, retailers, and farmers. Any reduction in eFCR and mortality translates to improved fish health and welfare, reduced waste, and a lower environmental footprint through more efficient use of resources. Predictability improvements translate into greater control over outcomes and more reliable business. Furthermore, a reliable uptick in the superior quality at harvest and reduced melanosis and blood spots in salmon flesh mean stable, higher average selling prices along with customer satisfaction and trust. New sources of Omega-3 fatty acids help to ensure nutritional quality of feed whilst de-risking growth of the industry by diversifying the raw material basket.

## References

This study builds on earlier work published in Frontiers of Marine Science Vol.11 2024: https://doi.org/10.3389/fmars.2024.1379066

# FEEDING RESPONSES OF WHITE-LEGGED SHRIMP (*Penaeus vannamei*) TO VARYING RATIOS OF BREWERY SPENT GRAIN (BSG) AND YEAST (BSY) WITH OR WITHOUT BSG SPONTANEOUS FERMENTATION

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## Introduction

The cost and availability of protein ingredients is a challenge for the development of the aquaculture feed sector, especially in developing areas. As a result, ingredient such as brewery spent grains (BSG) and yeast (BSY) constitute an interesting option. BSG has been the focus of several nutritional studies since the 90' with various results (1). The high-water content (75-85%) of BSG when exiting the brewery limits its use due to the heterogenous mastering of drying process in factories (2). Then, spontaneous fermentation as a low-cost conservation method seems to be an interesting opportunity. BSY have been widely studied for their role in boosting immunity. However, their interest as a palatability and attractivity enhancer remains little studied. Therefore, this trial aims to determine whether the fermentation state of BSG influence feeding behaviour of shrimp depending on the BSY/BSG ratio.

## Material & methods

The behavioural trial aims to study whether feed attractivity and palatability are influenced by the state of fermentation of BSG and by the ratio BSG/BSY following a factorial design described in Table 1. 35 shrimp will be distributed in 7, 50L reserve tanks with 5 shrimp per tank. 7 isoproteic (17,4%) experimental feeds containing a neutral base and 30% of BSG and BSY mixture will be crafted. During 5 days, shrimp will be presented by group of five to the feeds in a tank dedicated to behavioural studies equipped with cameras. Each group is only presented to a feed once a day and is never presented to the same feed twice.

Recorded videos will then be analysed with an AI based tracking software to generate data on palatability and attractiveness based on parameters such as prehension speed, time spent in the feeding zone, shrimp activity. Feed consumption will also be monitored. Data will then be analysed via statistical analysis.

## Objectives

Experiments assessing ingredient attractivity and palatability took place in April 2025. The growth trial started the end of April 2024 and will end in June 2025. These experiments will help to fill the data gap while providing information on the interest of BSG and BSY for aquafeed.

Table 1. Behavioural and growth trials factorial design

| | Non fermented BSG | Fermented BSG |
|---|---|---|
| 90% BSG / 10% BSY | r=5 | r=5 |
| 70% BSG / 30% BSY | r=5 | r=5 |
| 50% BSG / 50% BSY | r=5 | r=5 |

| Control |
|---|
| r=5 |

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# METHODS IN NUTRITIONAL RESEARCH IN *Penaeus vannamei*: A META-ANALYSIS OF RECENTLY PUBLISHED SCIENTIFIC PAPERS

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Improving the sustainability of *Penaeus vannamei* farming relies on a better understanding of its nutritional requirements, enabling the development of feeds and feeding strategies that enhance productivity while reducing environmental impact. This meta-analysis compiles data from 45 studies published in 2024-2025 across 4 scientific journals: Aquaculture International, Aquaculture Nutrition, Aquaculture Research, and Aquaculture. The aim is to compare the conditions under which nutritional trials are performed and assess their potential impact on P. *vannamei* performance.

A rigorous selection process was applied to include only studies dealing with feeding rate (FR), stocking density, frequency of daily feed distribution, considered as the parameters directly influence shrimp performance, and their effects on key performance indicators such as specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR). Spearman correlations and regressions quantified and illustrated these effects on growth.

Results indicate heterogeneity in experimental conditions, impacting growth performance outcomes. The average trial duration was 51 ±14 days. Similarly, initial body weight (BW) varied widely with a mean of 2.5 ±2.35 grams. Stocking density also exhibited significant variation, averaging 158 ±109 shrimp per $m^3$. Most studies apply 3 to 4 daily meals, with daily FR of 6 ±2 % of the biomass.

SR, SGR and FCR all exhibited high variability across studies. SR fluctuates significantly, with a mean of 85.5 ± 10.7%. SGR varied considerably, with an average of 3.87±2.12 % per day. Similarly, FCR also showed notable dispersion, averaging 1.63 ±0.39, reflecting variations in feed efficiency and experimental setups.

This meta-analysis shows a strong positive correlation ($r = 0.78$) between FR and SGR, confirming the fundamental role of nutrition in growth performance.

This study reveals significant heterogeneity in experimental protocols, impacting shrimp performance assessments. To address this, we established benchmarks to facilitate inter-trial comparisons. Establishing common research frameworks would facilitate meaningful performance assessments and improve the applicability of nutritional findings to shrimp farming. Future work will integrate water quality for better models and efficiency.

# ASSESING FISH WELFARE TROUGH LESS-INVASIVE HORMONAL DETERMINATION

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**Introduction**

Society is increasingly aware of animal welfare, and understanding the stress animals' experience due to their farming conditions is becoming critical. Stress can be defined as a state of threat to the organism homeostasis, which tries to restore itself by activating a complex repertoire of behavioral and physiological processes that are integrated in its adaptive response. This response primary involves the synthesis and secretion of adrenal glucocorticoids. In both, fish and humans, cortisol is the main glucocorticoid and its plasma levels are commonly used as an indicator of the acute stress levels (Sadoul & Greffoy, 2019). However, prolonged exposure to stressors induces desensitisation of the HPI axis to the stressor as a result of allostatic overload (Agulleiro et al., 2013; Aerts et al., 2015). Recent studies have shown that structures such as hair, feathers or scales accumulate cortisol and hormone levels could therefore be a good indicator of cumulative or chronic stress or, in other words, an excellent way of obtaining a history of non-specific stressful events (Aerts et al. 2015). Our studies show that chronical stress significant increase scale and fin cortisol in Mediterranean farmed species such as sea bass, sea bream and meagre supporting its use as potential indicator of accumulative stress in fish.

**Materials and methods**

Animals: Sea bass (*D. labrax*), sea bream (*S. aurata*) and meagre (*A. regius*), weighing aprox 200g, were kept in 500-litre tanks at 18 °C with a 12-hour light/dark photoperiod and fed a commercial feed. Prior to handling, the fish were anaesthetised with tricaine and euthanised with an overdose of the same anaesthetic.

Mechanical stress: The fish were divided into two groups (control and stressed). The stressed group underwent the stress protocol three times a week for one month by lowering the water level in the tank and exposing the fish to air for one minute. The control group was not subjected to any stressors. Finally, blood, scales, and fin (pectoral and caudal) samples were extracted from 12 animals per group to evaluate cortisol levels.

Cortisol levels in serum, scales and fins: Cortisol levels in the different tissue matrixes were evaluated by ELISA (Cayman) according to the manufacturer's instructions. Scales and fins were washed three times in saline phosphate buffer and incubated O/N in methanol that was subsequently evaporated. The samples were then resuspended in acetate buffer and purified using chromatographic columns (Superclean L-18 SPE, Sigma) prior to assay.

**Results and discussion**

The stress-inducing protocol was evaluated in previous studies (Leal et al., 2011). In all three species, cortisol levels significantly increased in various tissue matrices, including scales, pectoral and caudal fins, following the stress protocol. However, hormonal levels in scales were much lower than in other tissues. Plasma cortisol levels were also elevated in all three species. Seabass exhibited the highest hormonal levels in all four tissue types, which suggests that a higher hormonal response leads to greater cortisol accumulation in peripheral structures. Previous studies have reported the accumulation of cortisol in the scales of sea bass and goldfish following stress protocols (Laberge et al., 2019). However, our results show that the caudal and pectoral fins accumulate higher levels of cortisol following long-term stress protocols, thus supporting their use as excellent matrices for long-term stress monitoring. We also demonstrate that this hormonal accumulation extends to other species reared in captivity, such as seabream and meagre.

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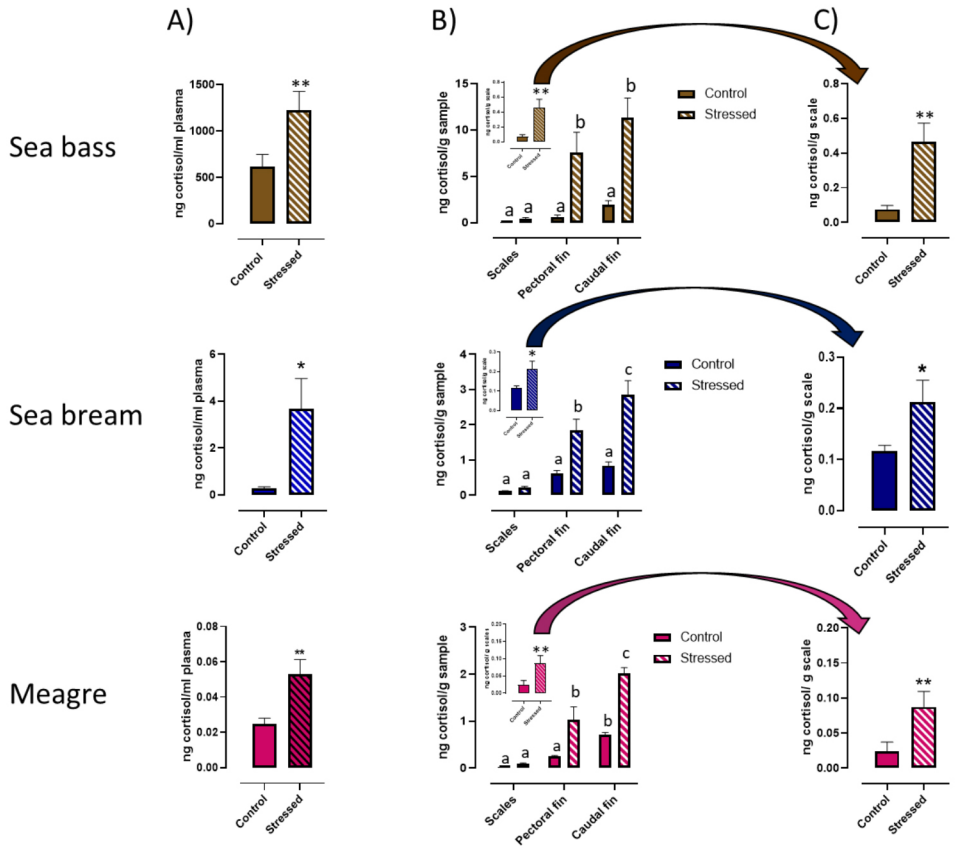


**Fig. 1. Determination of cortisol levels after physical disturbance imposed in sea bass, sea bream and meagre.** A) Quantification of cortisol plasmatic levels. B) Cortisol levels in scales and fins. Different letters denote significant differences after Two-way ANOVA ($p< 0.05$) and asterisk significant differences after *T-student* test. (*$p<0.05$, **$p<0.01$). C) Levels of cortisol in scales. Asterisk indicates significant differences after *T-student* test. (*$p<0.05$, **$p<0.01$).

## Conclusions

- Sea bass has the highest cortisol levels, followed by sea bream and meagre.
- Mechanical induced stress for 30 days significantly increases cortisol levels in serum, scales and fins (pectoral and caudal) in all three species.
- The caudal accumulated the highest glucocorticoid levels after long-term stress protocols.

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# AUTOMATION OF MARINE WATER QUALITY MEASUREMENTS: ANALYSIS OF METROLOGICAL ASPECTS

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## Introduction

Water monitoring, with high spatial and temporal resolution, is important to maintain its quality. In fact, in addition to the environmental damage that can be irreparable, the costs of pollution remediation are often higher than the cost of prevention or early intervention. Research must be directed towards proposing economically sustainable systems and procedures; only in this way it is possible to hypothesize an extensive use of water quality control and management systems. In order to create a qualitative/semi-quantitative water monitoring system, since it is impossible to monitor every specific pollutant, it is essential to establish an Early Warning criterion. In other words, we need to establish which parameters to measure and how to derive a synthetic pollution index from them that activates the rapid alert system. It is essential that the correlations between parameters must be identified in order to identify potentially redundant variables. To establish the Early Warning, it is useful that alarm thresholds are not "individually" established for each of the monitored parameters. In fact, it would be useful to identify a numerical index that can validly and correctly characterize the phenomenon from both a qualitative and possibly quantitative point of view. In general, the requirements that a numerical "alarm" index must have are mainly two:

a. relevance of the index components to the identification of the phenomenon in a significant and unequivocal way;
b. validity of the index limited within a well-defined scope and/or numerical interval, identified by pre-established minimum and maximum values.

In order to define a syntetic index that allows to generate an Early Warning, the paper explores metrological aspects that should allow to define a minimal set of physical/chemical parametrs to monitor.

## Laboratory Water Quality Testing

In order to verify the theoretical approach a series of test have been performed in laboratory using a suitable and calibrated multiparametric probe (Fig. 1): the SMx-485 of GEOVES s.r.l.. The probe has been inserted as sensor in a new automatic system able to analyze the quality of the water and to store samples of water in bottles if the Early Warning is generated. The system has been designed aiming to semplicity, reliability and robustness so allowing it to be mounted even on rovers as MAR – Multipurpose Amphibious Rover, which does not offer an environment particularly comfortable.



Fig.1. SMx-485 of GEOVES s.r.l.

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# ENHANCED RESILIENCE TO *Tenacibaculum maritimum* IN ATLANTIC SALMON SUPPLEMENTED WITH A NOVEL YEAST-BASED INGREDIENT

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## Introduction

Farmed Atlantic salmon are frequently exposed to infectious and non-infectious factors as well as necessary management practices causing serious mucosal health issues. Functional feeds are widely used as preventive measures to enhance fish health and well-fare among which yeast cell wall (YCW) products are well-established. We evaluated the potential benefits of in-feed supplementation with specific YCW products against *T. maritimum* tenacibaculosis which causes severe ulcerative lesions at mucosal surfaces and mortalities.

## Materials and Methods

A base-feed recipe was formulated according to current practices in the European Atlantic salmon industry. The trial tested 3 diets in quadruplicate: The base diet either non-supplemented (Control) or supplemented with one of two novel YCW products (Proto or Y+ respectively; Lallemand, France). Fish were fed to apparent satiation over the trial's duration. The trial lasted 15 weeks with fish exposed to *T. maritimum* by immersion end of week 6. Growth, feed performance and survival were monitored; skin and gill mucosal barrier were assessed; development of the disease was quantified by visual scoring of the gross skin and gill lesions at frequent interval post-challenge.

## Results and Conclusion

Compared to the Control; Y+ or Proto significantly improved survival (65.6% and 62.1% compared to 50.1%; +31.1% RPS for Y+; Fig A). However, only Y+ further improved the profile of skin lesion severity at end-point (Fig B). Analyses of histology, gene expression and mucus qualities are on-going to decipher the beneficial effects of the YCW product on mucosal robustness and disease resilience.

**In conclusion**, the novel-YCW product Y+ has the potential to contribute to the preventive management of tenacibaculosis in seawater Atlantic salmon.

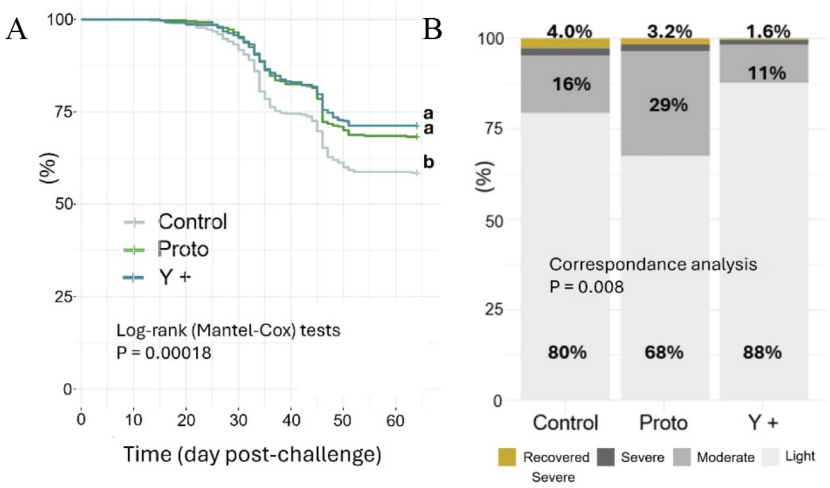


**Figure 1:** A) Survival profile and B) proportion of skin lesion category per diet at the end of the trial. Different letters indicate significant differences between diet.

# ROLE OF INTERLEUKIN-6 IN *Edwardsiella piscicida* INFECTION REVEALED THROUGH CRISPR/CAS9 GENE EDITING IN ZEBRAFISH

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## Introduction

Interleukin-6 (IL-6) is a key cytokine that orchestrates immune responses and is widely recognized for its roles in promoting inflammation, regulating cell survival and apoptosis, and providing protection against bacterial and viral infections. Previous research using *il-6* knockout mice has highlighted its importance in bacterial defence; these mice exhibited lower survival rates and impaired bacterial clearance. While the immunological functions of IL-6 have been well characterized in mammals, its roles in teleost remain unclear. In this study, we utilized zebrafish (*Danio rerio*) to investigate the significance of IL-6 in antibacterial defence against *Edwardsiella piscicida (E. piscicida)*. *E. piscicida,* a gram-negative bacterium of the Enterobacteriaceae family, is known to pose major threats by causing organ damage, ulceration, and eye abnormalities, often resulting in high mortality of various freshwater and marine organisms. We employed CRISPR/Cas9 gene editing technology for comparative analyses between wild-type (WT) and *il-6* knockout (*il-6* $^{-/-}$) zebrafish.

## Materials and methods

Utilizing the CRISPR/Cas9 technology, we constructed an *il-6* $^{-/-}$ zebrafish line. Both WT and *il-6* $^{-/-}$ fish were challenged with a low ($1x10^7$ CFU/ml) and high ($1x10^8$ CFU/ml) concentration of *E. piscicida* though immersion. To assess the host's susceptibility to the pathogen, we monitored cumulative mortality up to 11 days post-infection (dpi) and measured the amount of *E. piscicida* gDNA within the fish. Lastly, expression patterns of immune related genes such as pro-inflammatory cytokines (*il-1β*, *tnf*-α), signalling molecules (*stat3*, *socs3*), and antimicrobial peptides (*mpx*, *mpeg*) were observed through RT-qPCR.

## Results

A 7 base pair deletion was formed in the *il-6* sequence, resulting in a knockout of the gene which yielded a non-functional IL-6 protein. The genetic alteration led to dysregulated expression of immune related genes. These fish showed significantly elevated expression levels of proinflammatory cytokines, which coincided with a downregulation of the negative immune regulator *socs3*. Meanwhile, antimicrobial peptides were suppressed. The *E. piscicida* challenge assay revealed an increase in susceptibility to *E. piscicida* infection in knockout fish. These fish showed significantly higher mortality rates with earliest deaths occurring at 3 dpi. This heightened vulnerability corresponded with higher bacterial loads, indicating a flawed pathogen clearance. After the bacterial challenge, immune related gene expression in *il-6*$^{-/-}$ fish mostly peaked at 24 hours post-infection, although *il-1β* and *mpx* showed delayed expression peaks at 48 hours. Overall, the altered expression profiles of key immune genes indicate a disrupted antibacterial signalling network in the absence of IL-6. These findings underscore the critical role of IL-6 in initiating and coordinating immune responses following bacterial infections in zebrafish.

# LONG-TERM FISH MEAL REPLACEMENT WITH ANIMAL AND PLANT PROTEINS IN EXTRUDED PELLET DIET FOR OLIVE FLOUNDER *Paralichthys olivaceus*

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## Introduction

Fish meal (FM) remains a primary and costly protein source in formulated fish diets, due to its high nutritional value and excellent palatability. The global FM production has decreased by a yearly average of 1.7% since 1995. Animal or plant protein sources have been investigated as potential substitutes for FM. Olive flounder is a carnivorous marine species and the most cultured species in South Korea. In 2024, olive flounder production reached 40,125 tons accounting for approximately 50% of the total finfish production. Therefore, this study was conducted to evaluate the effects of FM replacement with combinational animal and plant protein sources in extruded pellet diet for the fish through a long-term feeding trial evaluation.

## Materials and Methods

A control diet (F70) was formulated to contain 70% sardine and anchovy FM (1:1, v:v). Two other diets were formulated by reducing FM levels from F70 diet to 35 (F35) and 25% (F25), while incorporating increasing proportions of both animal proteins (poultry byproduct meal and tankage meal) and plant proteins (soy protein concentrate and wheat gluten meal). The replaced diets were supplemented with lysine, methionine, taurine and betaine to meet the requirements and to increase the palatability of the fish. Additional additives included choline, lecithin, vitamin C, vitamin E and monocalcium phosphate. Total 540 fish (average body weight: 178 ± 1 g) were stocked into nine 900-L polypropylene tanks (60 fish per tank) in triplicates per dietary group. The fish were fed two times daily (08:30 and 17:30 h) until they were apparently satiated for 4 months.

## Results

After 4-month feeding trails, fish growth was not significantly affected by dietary FM levels (Table 1). Also, feed utilization, survival and biological indices were not significantly affected by the dietary FM levels. The results on immune-related gene expression and intestinal morphology will be further discussed in detail.

Table 1. Growth, feed utilization and survival of olive flounder (*Paralichthys olivaceus*) fed the experimental diets for 4-month.

| Diets | FMBW (g) | WG (%) | FCR | Survival (%) |
|---|---|---|---|---|
| F70 | 319±19 | 80±12 | 1.38±0.18 | 97.2±3.5 |
| F35 | 312±23 | 74±11 | 1.44±0.24 | 94.4±5.9 |
| F25 | 315±33 | 77±18 | 1.41±0.32 | 94.4±5.4 |

# SUSTAINABLE ALTERNATIVES IN MEAGRE AQUACULTURE: EVALUATING POULTRY BY-PRODUCT MEAL AS A FISHMEAL SUBSTITUTE

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**Introduction**

The aquaculture sector faces the challenge of balancing the growing demand for high-quality fish products with the need to reduce pressure on wild fisheries (Fao, 2018). As feed represents the highest operational cost in aquaculture, identifying alternative protein sources that are both cost-effective and environmentally sustainable has become a top priority. Processed animal proteins (PAPs) emerge as promising options, combining high nutritional quality with a low environmental impact, fully adhering to the principles of the circular bioeconomy (Galkanda-Arachchige *et al*. 2020). Meagre (*Argyrosomus regius*), a first-class whitefish species once abundant in the Adriatic Sea, has gained renewed interest in Mediterranean aquaculture due to its quality, rapid growth rate, and adaptability to farming conditions (Kružić *et al*. 2016). This study investigates the potential of poultry by-products (PBM) as a fishmeal substitute in meagre diets. Developing more sustainable feed formulations for meagre is crucial to support the aquaculture industry in producing these species in a way that maintains economic viability and environmental responsibility, without compromising product quality. Sustainable feed innovations not only help reduce reliance on marine resources but also enhance the long-term resilience and profitability of the sector (Tacon and Metian, 2015).

**Material and methods**

Three experimental diets were formulated to meet the nutritional requirements of meagre, each incorporating PBM as a partial replacement for fishmeal at different inclusion levels. One control diet, containing fishmeal as the primary protein source, was also included. The feeding trial was conducted in an open-circuit, outdoor water system at the Laboratory of Aquaculture, Institute of Oceanography and Fisheries (Split, Croatia). A total of 110 meagre (initial mean body weight: 1220 ± 154 g), obtained from a local farm, were acclimated for four weeks on a commercial diet before the experiment. Fish were then randomly distributed into six concrete tanks (600 L), with 18 fish per tank and two replicate tanks per dietary treatment. Growth performance was assessed through bi-monthly biometric measurements throughout the trial, while final evaluations of feed conversion ratio (FCR) and gut bacterial community composition will be performed at the conclusion of the experiment to comprehensively determine the impact of different dietary treatments.

**Results and discussion**

Our preliminary results from bi-monthly biometric measurements indicate that PBM holds significant promise as an alternative protein source in meagre diets. Fish fed with PBM-inclusive diets showed no compromise in growth performance or overall fitness compared to those receiving traditional fishmeal-based diets. These findings suggest that PBM can effectively support the rapid growth rate and adaptability characteristic of meagre in aquaculture settings. Moreover, economic analysis revealed that PBM diets were the more cost-effective than fish-meal based diets, exhibiting the lower estimated production costs. This positions PBM as not only a nutritionally viable but also an economically sustainable solution, aligning well with the industry's goals to balance cost efficiency with sustainability. By reducing dependence on fishmeal and fish oil, PBM-based diets contribute to alleviating pressure on wild fisheries while supporting the circular bioeconomy principles. As our experimental feeding trial approaches its final stages, ongoing monitoring will provide further insights into the long-term impacts of PBM on health parameters and product quality.

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**Acknowledgments**
The authors are thankful to all members of the Laboratory of Aquaculture in Split for their help during the feeding trial and sampling. Support for this study has been provided by the project SustainAqua funded by Next Generation EU-National Recovery and Resilience Plan 2021–2026.

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# SOLID PHASE IN-LINE DENITRIFICATION IN MARINE RAS USING BIODEGRADABLE PLASTIC (FORZEAS™)

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For recirculating aquaculture systems to be environmentally sustainable nutrient wise, removal of N at the RAS or at the end-of pipe treatment is required. In-line denitrification configuration has advantages such as allowing more intensive use of water, reducing heating/cooling as well as a reduced usage of makeup water. In-line denitrification systems are not frequently used due to their complexity on the addition of carbon sources (methanol, ethanol) that can hamper the RAS water quality. Solid-phase denitrification could be a safer alternative technology, as carbon sources are not dosed in the reactor, but rather bacteria harvest the carbon on demand from the solid phase material (Forzeas™).

The application of solid-phase in-line denitrification in a marine RAS rearing rainbow trout was evaluated at different hydraulic retention times (5.6, 3.2 and 1 hour) for 117 days, using Forzeas™ biodegradable plastic as material in a serial configuration reactor. The solid-phase in-line denitrification reactor showed the highest N removal capacity (411 gN/m$^3$ Forzeas™) at HRT 1 hour with a removal efficiency of 40% NOx entering the reactor. The usage rate of the reactor at evaluated conditions during the 117 days trial was 2.7 kg Forzeas™ /kg N considering an entering oxygen concentration of 7 mg/L in the RAS system.

The configuration of the reactor allows low energy consumption (0.4-0.6 w/kg N), no addition of suspended solids to the RAS and a minimal operational dedication. Presenting an easy, efficient and sustainable technology for in-line denitrification.

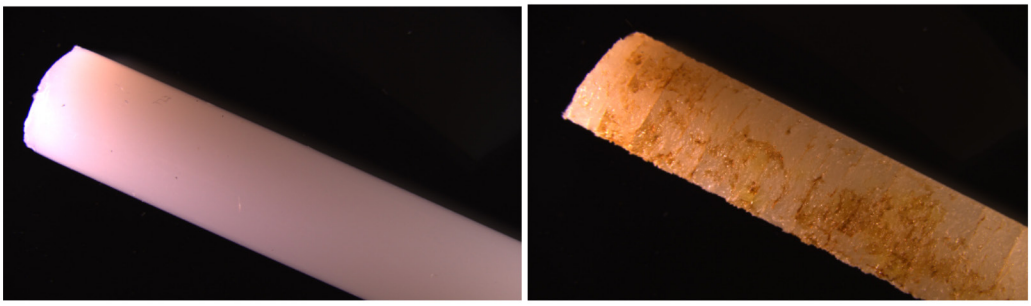

Figure 1: Biodegradable plastic FORZEAS™ before and after trial evaluation (117 days) in a Marine recirculating aquaculture system.

# TILAPIA GROWTH PERFORMANCE IN COUPLED AND DECOUPLED AQUAPONICS INCORPORATING A TRI-TROPHIC CYCLE

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**Introduction**

Aquaponics, which is a symbiotic integration of recirculating aquaculture and hydroponics constitutes an exemplary farming technique for water and nutrient recycling in a closed-loop system, thereby diminishing nutrient waste, water consumption, and land use. A critical evaluation of the coupled aquaponic system reveals that, despite its sustainability benefits, it faces certain limitations when compared to conventional cultivation methods, generally associated with decreased nutrient availability in the fish water (Tsoumalakou et al. 2022). The sustainability of the aquaponics system is also questioned in terms of aquafeed production, which is the only source of nutrients in the fish-crop system (Shaw et al. 2022). The primary protein component of conventional aquafeed is fish meal, which is mainly produced from wild fish populations. A promising alternative protein source for aquafeed is insect meal (Shaw et al. 2022).

In the present study a circular tri-trophic system is introduced which incorporates three organisms, plants, fish and insects, which feed each other. The black soldier fly (BSF, *Hermetia illucens*) larvae are used to produce insect meal, which is incorporated in the fish feed, the fish excrements give essential nutrients to the plants (aquaponics), and closing the circle, the plant residues (old leaves or other pruning material and fruits) feed the larvae of BSF. This system is designed to implement the circular economy, transforming waste from one organism into a resource for the next.

In this presentation, the fish growth results, aquaponics water physicochemical parameters and crop productivity/fish biomass gain indices are reported, concerning two consecutive experiments. These were performed with the crops being the only organism varying in the system; a cucumber-tilapia co-cultivation was performed in a three-month period during autumn, and a lettuce-tilapia in a 45-day experiment during the winter.

**Materials and Methods**

The experiment was conducted in the pilot-scale aquaponic greenhouse of the University of Thessaly, located in Velestino (39◦44′ N, 22◦79′ E), Central Greece. The total area occupied by the aquaponic facility is 432m$^2$ and is divided into the hydroponic subsystem (352 m$^2$) where crops are grown and the closed chamber (80 m$^2$) where the rearing of fish takes place (RAS), under controlled environmental conditions. Two aquaponics treatments were implemented: a) coupled aquaponics, in which the crops were irrigated only with fish-derived water and b) decoupled aquaponics, in which the fish water was enriched with fertilizers to reach the target of hydroponic cultivation of the specific crop.

The RAS system consisted of three fish rearing tanks of 1.5 m$^3$ water capacity, the mechanical filter that withholds solid fish excrements and the biofilter occupying nitrifying bacteria, which are an essential system component transforming the ammonia-rich fish excretion to nitrates for plant absorption. Temperature pH, EC and dissolved oxygen (DO) sensors were placed in the fish tanks for monitoring critical quality characteristics of the water (pH/EC/O2-measuring transducer, GHM-Greisinger, Regenstauf, Germany). Red tilapia (*Oreochromis* spp.) fish were reared in the RAS. The fish in each tank were fed at a rate corresponding to 1% of their total body weight daily. Insect meal derived from the insects farmed on-site comprised the 10% of the fish feed. The physicochemical parameters of the water in all fish tanks were measured daily; pH, and EC were measured with a portable sensor (HQ40d, Hach, Loveland, CO, USA). Temperature was maintained at 23 (± 0.4) $^o$C. After the conclusion of each experiment, the final fish biomass was determined and the weight gain, the Specific Growth Rate (SGR) and Feed Conversion Efficiency (FCR) indices were calculated. Finally, the production efficiency of the whole system was evaluated by the crop productivity/fish biomass gain index, calculated as the ratio of kg of product resulted from each aquaponics treatment to the fish biomass gain.

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**Results**

The physicochemical characteristics of the fish water were similar in the cucumber and lettuce experiments, with the exception of phosphorus, potassium and sodium concentrations, which were elevated in the lettuce setup.

The fish biomass gain in cucumber experiment was 42.3 kg, while in lettuce was 17.9 kg due to the shorter cultivation period, with the initial weight being 117.8 kg and 108 kg, respectively. SGR was also similar, reaching 0.4 % $day^{-1}$ in cucumber and 0.44 % $day^{-1}$ in lettuce experiment. The latter showed greater FCR values than cucumber, i.e. 2.12 and 1.56, respectively. Concerning the crop productivity/fish biomass gain index, the differences between the two aquaponics treatments were remarkable. DCAP exhibited greater values in both experiments in comparison with CAP, succeeding a 254% increase in cucumber and 179% higher values in lettuce.

**Discussion and conclusion**

The two reported experiments resulted in similar SGRs, which were low compared to other studies (Tsoumalakou et al. 2022), however the FCR was considerably higher than the values reported in these works. The ratio of crop productivity/fish biomass gain indicates the productivity of crops in comparison to aquaculture subsystems. According to our results, with the production of 1 kg of tilapia fish, 2.7 kg of CAP cucumbers or 9.6 kg of DCAP cucumbers were produced. If lettuce was cultivated only in CAP, 1.6 kg of lettuce would be produced for 1 kg gain for tilapia, while if only DCAP was implemented the result would be 4.5 kg of lettuce per kg tilapia gain. This difference is in accordance with other experiments using the same aquaponics variants (Aslanidou et al., 2024). Other studies calculated a crop productivity/fish biomass gain of 2.94 kg $kg^{-1}$ for lettuce cultivation and tilapia breeding with the supplementation of nutrients (Licamele 2009).

The DCAP variant of aquaponics systems is an effective system that enables the combined production of crops and fish, the latter being fed with sustainably derived insect protein.

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# SYNERGIC EFFECTS OF DIETARY NUCLEOTIDES AND VACCINE TO BOOST HEALTH OF EUROPEAN SEABASS *Dicentrarcus labrax* JUVENILES

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**Introduction**
Vaccination has significantly reduced antibiotic reliance in aquaculture; however, oil-adjuvanted injectable vaccines can cause undesirable side effects such as inflammation, peritoneal adhesions, and pigmentation. Dietary nucleotides (NT), essential molecules involved in nucleic acid synthesis, play critical roles in immune modulation, tissue repair, and metabolic regulation. This study investigated the potential benefits of combining a diet enriched with nucleotides alongside vaccination.

**Materials and Methods**
45 fish juveniles (initial weight: 51.54 ± 0.3 g) were assigned into one of four treatments: (N+Vac) Diet with NT + Vaccinated fish; (N) Diet with NT + Unvaccinated fish ; (S+Vac) Standard diet + Vaccinated fish; (S) Standard diet + Unvaccinated fish. After feeding the control or nucleotide (0.05%) supplemented diet for 12 weeks, the fish in treatment N+Vac and S+Vac were injected intraperitoneally with the ICTHIOVAC VR/PD vaccine (0.2 ml/fish). The fish from treatment N and treatment S did not receive any vaccine, but were injected with PBS (0.2ml/fish).

**Results and Discussion**
In 3-month feeding trial, comparing with fish fed standard diet, NT-fed fish showed increased lysozyme activity, total immunoglobulin (Ig), and total antioxidant capacity (T-AOC) in skin mucus, with T-AOC significantly elevated. In the head kidney, dietary NT significantly upregulated the expression of lysozyme (Lyz), immunoglobulin M (IgM), superoxide dismutase (SOD), catalase (CAT) and interleukin-10 (IL-10), while pro-inflammatory markerbinter leukin-1β(IL-1β) was significantly downregulated. Growth hormone (GH) expression was also enhanced, though no significant improvement in growth performance was observed. The addition of nucleotides enhances the fish's basic immunity and antioxidant status, contributing to improved farming resilience and health.

In 1-month vaccination trial, fish were sampled at the 2nd and 4th week post-vaccination. Comparing with S+Vac, N+Vac significantly modulated immune gene expression in the head kidney. Notably, the anti-inflammatory marker IL-10 remained elevated, and IL-1β suppressed at both time points, indicating sustained regulation of inflammatory responses. Expressions of Lyz and IgM were highest in the N+Vac throughout, suggesting prolonged enhancement of both innate and adaptive immunity. Antioxidant genes (SOD, CAT) were consistently upregulated in N+Vac fish, aligning with elevated SOD, peroxidase (POD), and T-AOC activities in skin mucus. Importantly, T-AOC remained significantly higher at 4 weeks post-vaccination, highlighting a prolonged antioxidant response. Bactericidal activity of mucus against *Vibrio harveyi* was also significantly enhanced in the N+Vac. Furthermore, NT supplementation alleviated vaccine-induced side effects such as peritoneal adhesions and pigmentation, particularly evident in week 3 (Fig 1).

**Conclusion**
These findings demonstrate that dietary nucleotides not only prime mucosal and systemic immunity but also extend the duration of immune enhancement post-vaccination, while mitigating inflammatory and oxidative stress responses.

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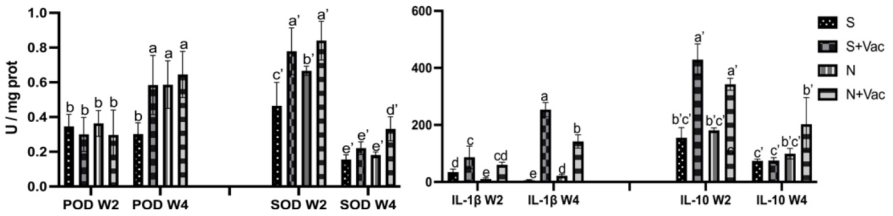


Fig. 1: European Seabass peroxidase and antioxidant activities in skin mucus and expression of inflammation-related genes in the head kidney at the 2nd and 4th week post-vaccination. The lowercase letters (a,b) represent significant differences in two-way ANOVA ($P < 0.05$) among the treatment and time.

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# EFFECTS OF HEAT STRESS AND TRADITIONAL CHINESE MEDICINE ON THE GROWTH AND METABOLISM OF MUD CARP *(Cirrhinus molitorella*)

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**Introduction**

In the context of global warming, the temperature of global lake systems has shown a climbing trend of 0.34°C per decade. Freshwater aquaculture ponds, being smaller systems than lakes, have slower heat exchange and are likely to experience more pronounced warming. It is reasonable to predict that freshwater aquaculture ponds will show a similar or more extreme warming trend. As most fish are ectotherms, their metabolism and physiology are directly affected by environmental temperature. While increasing temperature within the optimal range (25-30°C for mud carp) can promote fish growth, temperatures exceeding their tolerance threshold place fish under heat stress, threatening their survival and productivity.

Under heat stress, fish metabolism requires significantly more energy. Heat stress induces increased production of reactive oxygen species (ROS) and heat shock proteins, diverting energy away from growth when feed intake remains constant. Consequently, farmers must either use more feed or extend aquaculture time, reducing profitability in commercial operations.

Mud carp, a key member of polyculture systems in South China, has substantial economic value in the Pearl River delta region. Products derived from mud carp, especially canned preparations, are highly favored by consumers. For sustainable mud carp aquaculture under warming conditions, innovative solutions addressing both fish welfare and economic viability are urgently needed. Traditional Chinese medicine (TCM) has long been used in aquaculture to promote growth and enhance immunity while remaining cost-effective with minimal environmental impacts. Recent research indicates that flavonoids and polysaccharides in dandelion (*Taraxacum mongolicum*) can enhance fish growth, antioxidant status, and immune responses. This study investigates whether dandelion can serve as a nature-based solution to mitigate heat stress effects in mud carp aquaculture.

**Method**

To investigate the potential of TCM to mitigate heat stress effects on mud carp growth and metabolism, 96 fish were cultivated at 30°C (control) and 34°C (heat stress). They were reared with or without dandelion extract prepared by mixing dandelion powder (2% w/w, TCM/feed) with distilled water. Each treatment group contained 24 fish. The weight of each fish was recorded every two weeks. After 2 months of dietary treatment, serum and tissues (liver, muscle, and gill) were collected for biochemical analysis to assess oxidative stress markers and antioxidant capacity.

**Result**

Based on the growth performance results, fish fed with dandelion exhibited 62.6% higher relative weight gain and 34.8% better feed conversion ratio under heat stress, reflecting improved growth and feed efficiency compared to those fed without dandelion. Additionally, the analysis of antioxidant enzyme activity in fish serum indicated that fish fed with dandelion had 58.3% higher activity of superoxide dismutase and 17% higher catalase activity compared to those that were not fed dandelion under heat stress. These results showed that dandelion-supplemented mud carp experienced alleviated oxidative stress caused by ROS accumulation. Therefore, dandelion serves as a promising nature-based solution to reduce heat stress effects in mud carp. This project offers a valuable approach for mitigating the impacts of global warming on aquaculture species to promote the sustainable development of the industry in a changing climate.

# NATURAL ANTIOXIDANTS REDUCE OXIDATIVE STRESS AND IMPROVE LARVAL DEVELOPMENT IN ZEBRAFISH (*Danio rerio*)

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## Introduction

Resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defence, oxidative stress is a critical challenge for fish (Hoseinifar et al., 2020). In aquaculture settings, and particularly in hatcheries, oxidative stress is a major concern during the early stages of fish development due to the high metabolic activity of the larvae and environmental challenges (Menon et al., 2023). Excessive ROS production triggers lipid peroxidation, protein misfolding, and DNA damage, leading to reduced growth, impaired immune function, and increased susceptibility to disease (Jia, 2025). To mitigate these adverse effects, natural plant-derived antioxidants are increasingly being used in the aquaculture industry due to their broad-spectrum ROS-scavenging properties, low toxicity, and sustainability compared to synthetic alternatives (Hu et al., 2025). This study investigated the effects of water exposure to natural antioxidants on the larval development of zebrafish (*Danio rerio*), a widely recognised model for developmental and toxicological research, and for studying oxidative stress (Chowdhury and Saikia, 2022). The experimental design focused on three key aspects: survival and hatching rates, morphometric development, and ROS modulation.

## Materials and Methods

Wild-type and transgenic *casper* strains were used. The transparent *casper* strain, which lacks melanocytes and iridophores, was used to allow accurate *in vivo* quantification of ROS without interference from skin pigmentation (Rajpurohit et al., 2023). Fish were exposed to six concentrations of a commercially available natural antioxidant formula (0, 2.5, 5, 10, 25 and 50 mg/L) obtained from Marfeed (NAOX**,** MIAVIT GmbH, Essen, Germany) from 24 to 120 hours post-fertilization (hpf). Each treatment group consisted of 300 fertilised eggs, divided into three replicates. Survival and hatching rates were monitored daily. Morphological characteristics (such as body length, yolk sac area, and swim bladder inflation) and ROS levels were assessed at 120 hpf in 30 and 10 fish per treatment, respectively, according to Camussi et al. (2024). ROS production was assessed using the fluorescent probe carboxy-H2DCFDA, both under basal conditions and after induction of oxidative stress using the pro-oxidant molecule tert-Butyl hydroperoxide (Naef et al., 2021). Statistical analyses were performed using the Mantel-Cox log-rank test for survival rate data and the ordinary one-way ANOVA multiple comparison test for hatching rate, morphology and ROS data.

## Results

The study showed that antioxidant exposure did not affect survival or hatching rates ($p > 0.05$). Treated larvae exhibited significantly increased body length and swim bladder inflation and reduced yolk sac area compared to controls ($p \leq 0.05$) at each tested concentration. Fluorescence-based analysis (Fig. 1) showed a significant reduction in both basal and TBH-induced ROS levels ($p \leq 0.05$) at the 25 and 50 mg/L doses.

## Discussion and conclusions

These findings suggest that the natural antioxidants tested are safe up to concentrations of 50 mg/L. Furthermore, exposure to antioxidants enhances larval development by promoting faster development and higher larval metabolism, in agreement with the literature (El Basuini et al., 2022; Shastak and Pelletier, 2023). The use of the *casper* strain provided clear evidence of ROS reduction, confirming the utility of these natural compounds in oxidative stress management. Given the conserved nature of antioxidant biology across species (Birnie-Gauvin et al., 2017), zebrafish serve as a rapid and cost-effective model for aquaculture research. This study highlights the potential of natural antioxidants as sustainable tools to improve larval growth and health. Further long-term studies are needed to evaluate the effects of water exposure and dietary supplementation with natural antioxidants on growth performance and disease resistance in commercially farmed species.

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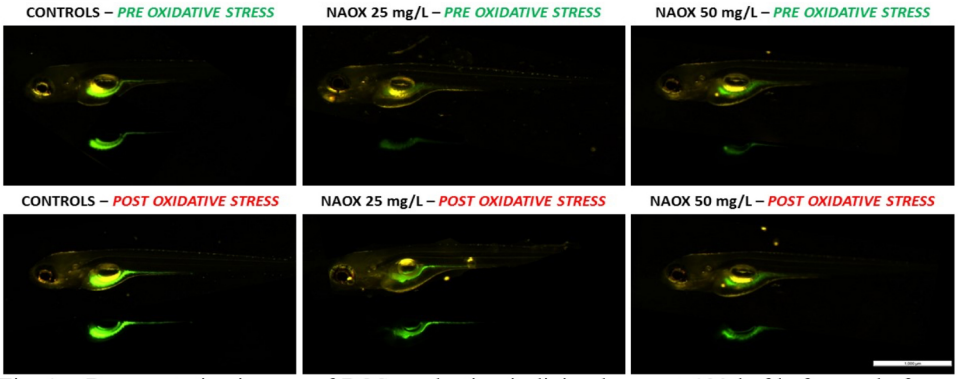


Fig. 1 – Representative images of ROS production in living larvae at 120 hpf before and after a standardised oxidative stress procedure. Within each image, the larva is visible under normal and fluorescent light at the top, and fluorescence only at the bottom. A 1 mm white scale bar is included.

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# SHORT-TERM STORED SPERM *IN VITRO* USED FOR BREEDING PROGRAM OF COMMON CARP (*Cyprinus carpio* L) IN CZECH REPUBLIC

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## Introduction

Global production of common carp increased from 1,205,000 t in 1990 to 4,360,000 t in 2020, with aquaculture accounting for the majority (97.2%). Within the European Union, the Czech Rep., Poland, and Hungary contributed 68% of the total common carp output (Shazada et al., 2025). The Czech Republic has maintained a selective breeding program for common carp for over 80 years. Over the past three decades, the program has adopted short-term sperm storage to enable controlled fertilization and preserve genetic diversity. In this context, sperm was collected individually and used at 0.5 to 5 days intervals depending on the breeding and/or testing protocols (Kocour et al., 2005). However, the use of short-term storage of carp sperm greatly reduced sperm fertility. Therefore, during the last five years, we have focused intensively on optimizing the method of sperm short-term storage. Firstly, to enhance the preservation of sperm motility and fertility, and secondly, to verify the potential effect of sperm storage on epigenetic changes in the offspring.

## Materials and methods

Mature males of carp were obtained following fish farm pond harvesting and maintained in a laboratory and hatchery aquatic system. Sperm and egg samples were collected individually after treatment with carp pituitary extract at 21°C. Sperm was diluted with carp extender (Cejko et al., 2018) and stored for 6-14 days at 0-2°C under aerobic conditions. Post-storage, 6 mL sperm suspension was warmed at 20 °C for 2 h, followed by assessment of spermatozoa motility, velocity, and hatchability. To investigate how sperm storage may affect offspring, we integrated DNA methylome, transcriptomic, and proteomic analyses. To standardize the protocol and minimize maternal variation, fertilization for the DNA study was conducted using eggs from a single female.

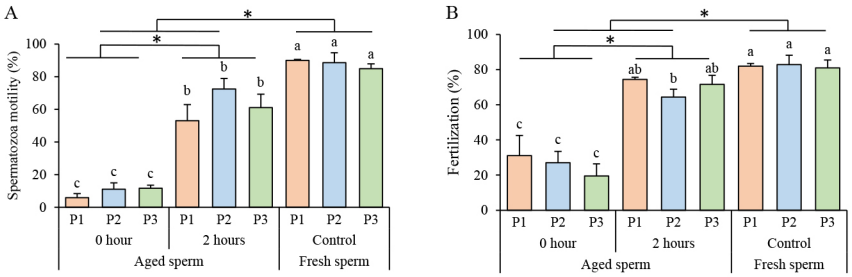


Fig. 1: Effects of warming temperature on motility and hatchability of large-scale short-term stored sperm 8 days at 0-2 °C (n=9; mean±S.D.) in carp under hatchery conditions (Zhang et al., 2023). Sperm from each of three males was pooled (6 mL total volume): males 1-3 (P1), males 4-6 (P2), and males 7-9 (P3) in 50 mL containers and incubated at 20 °C for 0 and 2 h. Fresh sperm was collected from the same males. Fertilization was performed using hatchery water at 30,000 spermatozoa per egg on 50 g eggs for each sperm pool. Values with different lowercase letters were different ($P < 0.05$). Values with asterisks showed significance ($P < 0.05$).

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**Results and discussion**

Carp sperm is always contaminated to some extent with urine (Billard et al., 1995). Although the level of contamination is low, storing sperm without an extender leads to a significant reduction in sperm motility and fertility within 24h, and sperm become immotile after 3 days post-storage (Cheng et al., 2022, Zhang et al., 2023, 2024). The most effective storage medium is the carp extender (110 mM NaCl, 40 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 20 mM Tris, pH 7.5, and 310 mOsmol/kg) (Cejko et al., 2018). When sperm is used for fertilization within 3h of sperm collection, it is not diluted and cooled to 0-2 °C but instead stored in the incubator at 15-20 °C. This is due to the high sensitivity of carp sperm to temperature (Zhang et al., 2023). Therefore, fertilization within three hours, cooling is avoided. In contrast, sperm intended for later use is diluted 1:1 with pre-cooled extender (Cheng et al, 2022) and stored at 0-2 °C without antibiotics, for up to 14 days (Zhang et al., 2023, 2024). Notably, dilution even after 3h post-collection can still maintain sperm quality, making this practice suitable for hatchery applications (Shazada et al., 2025). Stored and diluted sperm should always be warmed to 20 °C before insemination. A 2-hour warming period significantly improves motility and hatching rates (Fig. 1). Intriguingly, malformation rates increased, especially in fresh sperm compared to those fertilized with stored sperm without warming (Zhang et al., 2023).

Sperm storage negatively affected sperm motility, viability, and DNA integrity, but did not affect the global DNA methylation of sperm or embryos. However, whole-genome bisulfite sequencing revealed altered methylation patterns in differentially methylated regions (DMRs) associated with genes in embryos fertilized with the 6-day-stored sperm. Results provide strong evidence of epigenetic inheritance influencing offspring's performance following fertilization with stored sperm (Cheng et al., 2024). Therefore, in the carp breeding program, we aim to minimize the sperm storage duration.

**Funding information**

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# EFFECTS OF LYSOPHOSPHOLIPIDS AND BUTYRATE ON GROWTH PERFORMANCE AND HEPATIC LIPID METABOLISM RELATED GENE EXPRESSION IN LARGEMOUTH BASS *Micropterus salmoides* FED HIGH SATURATED FAT AND PLANT PROTEIN DIETS

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Limited availability, sustainability issue, and high cost restrict marine-derived ingredients utilization in aquafeed. Plant proteins and land animal fats are cost-effective alternatives but induce many challenges on growth and health. Specific plant oils and land animal fats contain high saturated fatty acids (SFA), which may lead to lipids accumulation. Lysophospholipids (LPLs) enhance nutrient absorption by promoting micelle formation and improving lipid transport. Butyrate, a short-chain fatty acid, supports intestinal health by serving as an energy source for enterocytes and reducing inflammation. Two studies were conducted to address issues posed by high SFA (fish stearin oil) and plant-based diets, offering potential strategies to optimize feed efficiency and mitigate metabolic disorders in fish. The first assessed LPLs' effects on growth and hepatic lipid metabolism-related gene expression, while the second investigated a combined LPL-butyrate approach.

First trial

The present study aimed to investigate the ameliorative effects of three inclusion levels of LPLs on fish stearin oil in largemouth bass. Five diets were formulated in a 68-days study, named WFO (130 g/kg winterized fish oil), FSO (130 g/kg fish stearin oil), LLO (FSO + 0.625 g/kg LPLs), MLO (FSO + 1.875 g/kg LPLs) and HLO (FSO + 3.125 g/kg LPLs), respectively. Largemouth bass fed FSO showed lower apparent digestibility coefficients (ADCs) of nutrients than the WFO. Medium and high doses (0.75, 1.25 g/kg) significantly increased ADCs of nutrients compared with low doses (0.25 g/kg) and FSO. Results showed significant higher FBW, FI, WGR and SGR in diet HLO than FSO, LLO and MLO. Diet MLO significantly decreased lipid and energy in whole-body and lipid retention compared with the FSO, LLO and HLO. In addition, activity and mRNA expression of ATGL significantly increased, while GPAT4 decreased in HLO (Figure 1).

Second trial

This study was conducted to evaluate the effects of lysophospholipids-butyrate (LPB) on a high plant protein diet in Largemouth bass. Three diets were formulated: positive control (PC) with 15.00 % soybean meal (SBM); high-plant-protein diet (HPP) with 28.00 % SBM; LPB diet supplement with 0.1 % LPB in HPP. Fish were fed for 60 days. Results indicated that the HPP group exhibited significantly lower FBW, WGR and SGR compared to the PC group. The HPP diet induced a notable increase in liver collagen fiber and liver lipid content, which was alleviated by LPB. In comparison to the HPP group, the LPB group showed significant decrease in alanine aminotransferase activity ($P < 0.05$). Compared with the PC group, the mRNA levels of ACC-1, DGAT, CPT-1 and TNF-α in HPP group were significantly increased ($P < 0.05$), while ATGL and HSL were significantly down-regulated. The mRNA levels of FAS, ACC-1, PPAR-α, ATGL, IL-1β and TGF-β were up-regulated ($P < 0.05$), while DGAT and TNF-α were down-regulated in the LPB group compared to HPP group ($P < 0.05$). In conclusion, the supplementation of 0.1 % LPB can alleviate liver lipid accumulation and fibrosis caused by high level of soybean meal.

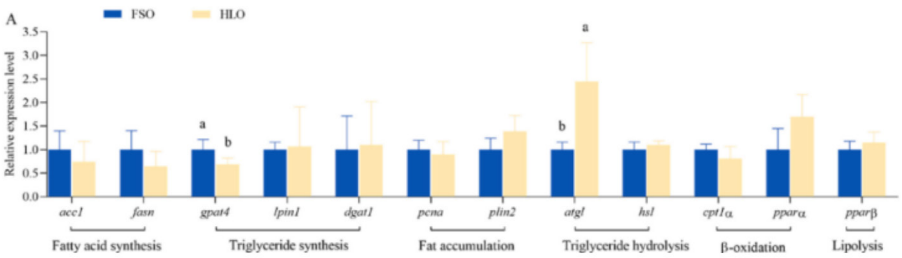


Figure 1, relative mRNA expression of genes involved in lipid metabolism

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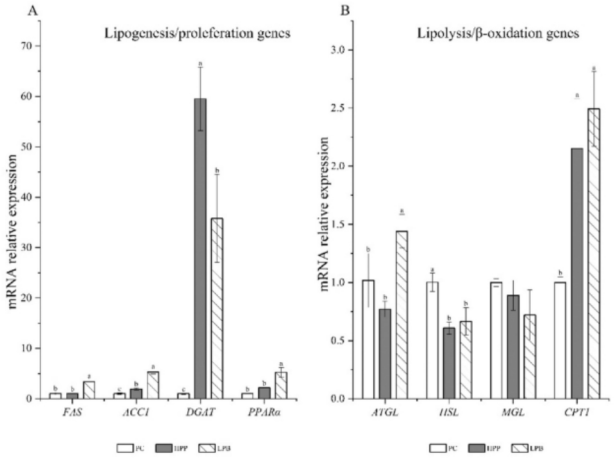


Figure 2, hepatic lipid metabolism gene expression.

Conclusion

The first trial revealed marked alterations in hepatic lipid metabolism-related gene expression between the control group and the lysophospholipids treated group. Notably, in second trial, the group supplemented with lysophospholipids-butyrate exhibited enhanced efficacy, demonstrating more pronounced differential expression of these genes compared to control.

# WITHDRAWAL TIME ESTIMATION AND DIETARY RISK ASSESSMENT OF SULFAMETHOXAZOLE IN GIFT TILAPIA (GIFT *Oreochromis niloticus*) AFTER ORAL ADMINISTRATION

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Sulfamethoxazole (SMZ), a widely used broad-spectrum antibiotic in aquaculture, lacks comprehensive research on its residual elimination kinetics in tilapia. This study investigated SMZ residue depletion, withdrawal periods, and dietary risks in 1-year-old GIFT tilapia (Genetic Improved Farmed Tilapia *Oreochromis niloticus*) weighing 500 ± 50 g, following oral gavage administration of a loading dose (200 mg/kg BW on day 1) then 100 mg/kg BW daily for 6 more days, at 22±2°C.Tissue samples (plasma, muscle, skin, liver, kidney, gill, and remaining tissues) were collected from 5 fish per time point at intervals from 0.33 to 30 days post-administration, with SMZ residues quantified via HPLC-MS/MS. Results revealed peak SMZ concentrations at 0.33 days (8 h), ordered as liver > skin > plasma > kidney > remaining tissues > gill > muscle. Muscle residues fell below the maximum residue limit (MRL, 100 μg/kg) by day 3, while skin required 10 days. Kidney residues dropped below the limit of detection (LOD) earliest (16 days), followed by muscle, gill, and remaining tissues (25 days), whereas plasma, liver, and skin retained detectable levels until day 30. Elimination equations for SMZ across tissues exhibited first-order kinetics. Based on the specific conditions of this study, a minimum 11-day withdrawal period is recommended for edible tissues (muscle + skin) after SMZ administration. Hazard quotient (HQ) values for all tissues remained below the safety threshold (HQ = 1), indicating low dietary risk. These findings support SMZ use standardization in tilapia aquaculture to ensure food safety compliance.

# INTEGRATING LOW-TROPHIC AQUACULTURE WITHIN OFFSHORE WIND FARMS: ASSESSING ECOLOGICAL IMPACTS THROUGH FOOD WEB MODELING IN THE GERMAN NORTH SEA

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Given the growing competition for marine space, the integration of low-trophic aquaculture (LTA) within offshore wind farms (OWFs) represents a strategic multi-use (MU) solution to enhance ocean productivity while safeguarding ecological integrity. This study examines the ecological impacts of cultivating extractive organisms such as *Mytilus edulis* and *Ulva spp.* within the Meerwind Süd | Ost OWF, located in the German Exclusive Economic Zone of the North Sea. While LTA can contribute to ecosystem services like carbon sequestration and bioremediation, large-scale implementation may also generate ecological trade-offs through nutrient competition, sedimentation, or altered food web dynamics. To investigate these effects, a food web model will be developed using Ecological Network Analysis, incorporating biotic and abiotic data. The model aims to (1) analyze material flows before and after MU implementation, (2) evaluate carrying capacity limits for sustainable LTA expansion, and (3) assess functional shifts in the OWF food web. A systematic literature review is being conducted to inform model structure and parameterization. The outcomes of this research will deliver critical early-stage insights into the feasibility, trade-offs, and ecological thresholds of integrating LTA in offshore energy infrastructures. By providing an ecological perspective, this work aims to inform evidence-based marine spatial planning, guide regulatory frameworks, and support the development of environmentally sound offshore MU initiatives in the North Sea and beyond.

# DIFFERENT SCENARIOS OF PHOTOTHERMAL MANAGEMENT AND ITS IMPACT ON THE OUT-OF-SEASON EGG QUALITY IN PIKEPERC (*Sander lucioperca)*?

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**Introduction**

The development of protocols for photothermal spawning induction in pikeperch has been a subject of interest for both scientific and commercial purposes during the last few decades (Hermelink et al., 2011; Żarski et al., 2019; Ljubobratović et al., 2025). Accordingly, several protocols have been published, showing their applicability on different levels of reproductive biology indicators. Nevertheless, the advanced gametogenesis cannot guarantee high reproductive performance, which is especially so in the case of eggs, where late stimulation of ovulation may lead to a reduction in gamete quality (Ljubobratović et al., 2022). Therefore, the evaluation of different scenarios of protocols for induction of out-of-season induction and their effect on egg quality upon hormonal stimulation is of importance for establishing indicators of the appropriate maturation stage for the procedure of artificial reproduction.

**Materials and methods**

The study was conducted in one climate chamber over two consecutive years in period from December 2020 to November 2022. In this manner, two photo-thermal spawning protocols were applied: SHORT - 19-week-long, composed of cooling only; LONG - 23-week-long, composed of 19 weeks of cooling with an additional four weeks of chilling. Accordingly, the first reproduction took place in early October 2021, while the second one was performed in early November 2022. For both reproductions, the warming thermal protocol of the artificial reproduction was conducted (Ljubobratović et al., 2021). The SHORT group was composed of 18 pairs of breeders, while the LONG group was composed of 15 pairs of breeders. The ovarian follicle diameter was measured three times during the cooling and chilling period, and egg and sperm quality were examined during the artificial reproduction.

**Results**

Significantly higher ovarian follicle diameters were found at each sampling moment in the LONG group. Latency time was significantly shorter in LONG groups, while main egg quality features were better in the SHORT group, except pseudo-gonadosomatic index, which was higher in the LONG group. On the other hand, better sperm quality was observed in the LONG group.

**Discussion**

Based on the assessed results, it seems that the final chilling phase, composed of a stable minimal temperature, is not required in case of artificial reproduction using external hormones for ovulation stimulation. While this phase appears beneficial for males, it may lead to latency time reduction and, consequently, to egg quality deterioration. Future studies should evaluate whether the low temperatures are necessary for out-of-season artificial reproduction.

**Acknowledgements**

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# LINKING CONSISTENT BEHAVIOURS TO PHYSIOLOGICAL STRESS RESPONSE IN ORDER TO UNDERSTAND FISH WELFARE INDICATORS

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I would like to present the preliminary results for boldness/exploration of the fall and the correlation with physiology results: glucose and cortisol. It looks from what we did with Maelenn that boldness and exploration are repeatable but not correlated with glucose (doesn't seem correlated with cortisol either from the couple graphs that I made). I might have time over the summer to do some more stats. If I don't find any correlation, it also opens the debate on: do the stress induced by netting and the boldness test induce a similar response? It also raises the question of repeatability of the cortisol and glucose measurements.

An increasing number of studies are investigating physiological, neural, morphological, or behavioural indicators of welfare in fish. Yet, the links between these traits remain poorly understood. Animal personality refers to consistent individual differences in behaviour across time and contexts. Studies have shown that several behavioural traits in fish, such as boldness and exploration, exhibit such consistency. In some species, these traits have been shown to correlate with fitness, and indicators of welfare. However, few studies have investigated the correlation between these traits. Consequently, the underlying mechanisms shaping fish personality remain unclear. To address this gap, we raised Arctic charr (*Savelinus alpinus*) from an Icelandic aquaculture strain, representing six different families, in either plain tanks or tanks containing a shelter. Tagged individuals were tested for behaviour three times in an open field test and in an emergence test respectively. Following the behavioural trials, the same fish were exposed to a standardized netting stressor, and blood samples were collected either 40 minutes or 2 hours 40 minutes post-stress. We first assessed the consistency of exploration and boldness. Then, we investigated the influence of genetic (family) and environmental (tank condition) factors on personality traits and physiological stress responses. Finally, we explored correlations between traits. This study highlights the relevance of understanding the physiological underpinnings of personality traits in relation to fish welfare. The project and its preliminary results regarding the factors influencing physiology and behaviour, as well as the correlations between these traits will be presented.

# LONG INTESTINES AND STRANGULATING OBSTRUCTION IN FARMED ATLANTIC COD *Gadus morhua*: A RESULT FROM ENVIRONMENTAL FACTORS AND FEEDING STRATEGIES?

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## Introduction

One of the main challenges for the Atlantic cod (*Gadus morhua*) farming industry is a fatal intestinal disorder that compromises animal welfare and causes high mortality. The production of Atlantic cod is experiencing a resurgence to fulfil market demand for a stable fish supply, and ambitions are high in expanding the industry for the future. The pathological gut conditions, undoubtedly painful for the fish, occur from small to large slaughter-size causing high economic losses and is considered a main obstacle for further increase in production. There has until now been a genetic selection process for growth, behaviour and other favourable domestication traits in Atlantic cod, but gut anatomy and gut health have so far not been the focus of the cod breeding programs. The current feeding strategy for cod is moreover based on knowledge and infrastructure from the salmon industry and is probably not yet optimally adapted for cod farming. The gut can show remarkable phenotypic flexibility in response to dietary and other environmental signals. The overall goal of the ongoing research project FôrCod (RCN project no. 337056) is to reduce mortality in cod aquaculture related to intestinal disorders, by increasing our understanding of cod gut anatomy, prevalence and extent of gut disorders, and identifying potential underlying causes of pathological conditions.

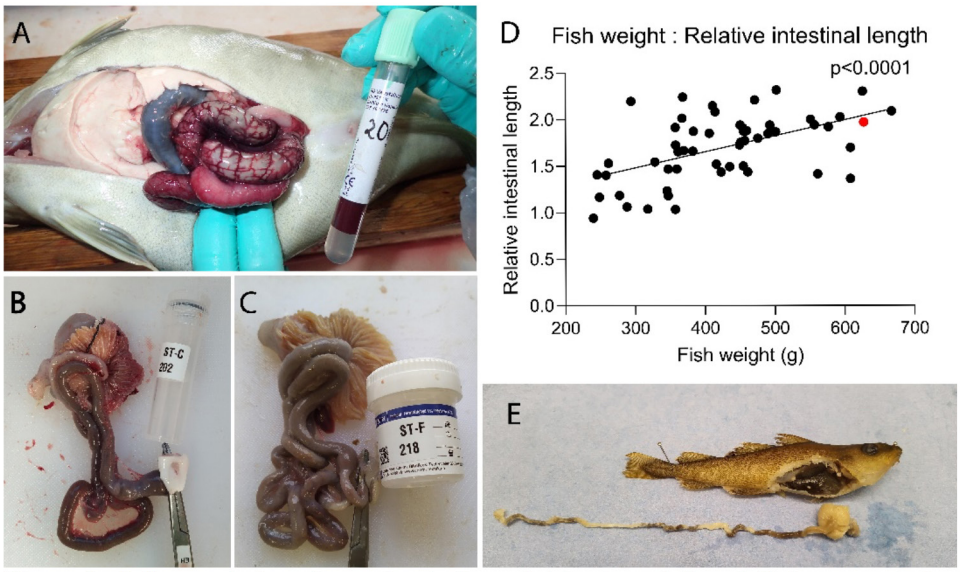


*Figure 1. A) Strangulating obstruction in farmed Atlantic cod. B and C) Large variation in intestinal length and organization in farmed cod. D) Relative intestinal length is linked to fish weight. The red dot illustrates an individual with strangulating obstruction. E) Long intestines in early developmental stages.*

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**Materials and methods**

Several field surveys and controlled feeding trials have been carried out to study factors to better meet the physiological requirements of Atlantic cod, including improvements of feeding regimes during on-growing stages (meal frequency, underwater feeding) and diet composition (ash and chitin content, moisture, raw material use). Field surveys in cooperation with commercial cod farmers have been performed to investigate the current status of the gastrointestinal system of farmed cod. Inclusion of crab shell meal in the diet have been tested, both in a controlled short-term digestibility trial [1] and in a large-scale feeding trial in practical production. Cod at early developmental stages, obtained from the cod breeding program of Nofima, have moreover been studied. Studies have focused on gastrointestinal anatomy and organization, gut morphology and health, and nutritional status.

**Results**

We recently published the first in-depth pathological description of the intestinal disorder, which is correctly denoted 'strangulating obstruction' [2] (***Fig. 1A***). In affected fish, part of the intestine is permanently entrapped (incarcerated) under fibrous strands in the mesentery. The entrapment interferes with blood flow and physically blocks the flow of the intestinal content, causing a strangulating obstruction with luminal fluid build-up, severe venous congestion causing ischemia of the intestinal wall.

We have observed a large variation in gut morphology observed in today's farmed cod (***Fig. 1B-C***) where relative intestinal length increase with fish size (***Fig. 1D***). Compared to wild cod, the farmed cod has much longer intestine and a more complex organization of the gastrointestinal tract and mesentery. Cod farmers report that intestinal disorders primarily manifest during periods of rapid growth and high feed intake and increase in severity as the fish grow larger. This suggests an association between the long, complicated intestines of the farmed cod and the occurrence of fatal strangulating obstruction. The long and complicated intestine seems to develop during early life stages of farmed cod (***Fig. 1E***) indicating that problems with intestinal strangulation can be rooted in the larval stage. Whether the complicated intestinal system in the farmed cod is related to the environmental conditions such as the feeding regime, a result of genetic breeding, or a combination, is not known. Results from several trials and field surveys performed as part of the project will be presented and discussed at the conference.

**Acknowledgements**

This project has received funding from Research Council of Norway (FôrCod project no. 337056).

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# “CONTRASTING MEDIA NARRATIVES ON AQUACULTURE IN SPAIN AND FRANCE”

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Aquaculture has gained increasing recognition as a key sector contributing to food security, economic development, and environmental sustainability. However, its expansion is influenced by societal perceptions, which are shaped by media representation. This study presents a comparative analysis of media coverage and societal perceptions of aquaculture in Spain and France, with a particular focus on the differing narratives surrounding the trout and salmon industries. By employing content analysis and sentiment classification, we assess how these sectors are portrayed in the media, the key themes associated with them, and the evolution of public discourse over time.

The methodology consists of a systematic media analysis, collecting and categorizing news articles related to aquaculture from national and regional sources. Sentiment classification was conducted to determine the portrayal of aquaculture as positive, neutral, or negative, while thematic content analysis identified key stakeholders, recurring narratives, and dominant concerns in the discourse.

Our findings indicate that in Spain, media coverage of aquaculture has intensified in recent years, with a growing emphasis on sustainability, innovation, and market development. While the perception remains largely positive (48% of articles), there has been an increase in neutral (26%) and negative (27%) portrayals, often linked to environmental concerns and regulatory challenges. In contrast, in France, discussions were more centred on traditional fish farming, particularly trout and salmon production, with a predominance of positive coverage (63%). However, issues such as policy constraints and environmental sustainability were also significant themes.

A notable divergence emerges in the media representation of trout versus salmon. In France, trout farming has been portrayed as a traditional and localized industry, benefiting from strong regional identity and consumer trust. In contrast, salmon aquaculture, particularly in relation to international markets, has faced greater scrutiny regarding environmental impact, disease outbreaks, and regulatory challenges.

In Spain, the commercialization of trout has been positioned as an innovative and growing segment of the market, while salmon remains a dominant but sometimes controversial sector, often linked to broader discussions on global sustainability and competitiveness.

This study highlights the importance of transparent communication and knowledge dissemination in fostering public trust and social acceptability of aquaculture. Understanding how media narratives evolve can inform policy strategies to enhance societal engagement and support the sustainable development of the sector.

# GONADAL SEXUAL DIMORPHISM IN GENOME-WIDE DNA METHYLATION AFTER BACTERIAL IMMUNE CHALLENGE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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## Introduction

In the current global population growth and climate change, there is an urgent need to supply the global fish demand while addressing key challenges in aquaculture, particularly those related to the immune response in farmed fish, which affects their vulnerability to infections. The European sea bass (*Dicentrarchus labrax*) is one of the most widely cultured species. According to APROMAR (2023), in 2021, this species ranked as the fifth most farmed in Europe and has shown a steady increase in production, rising from 82,825 tonnes in 2020 to 96,647 tonnes in 2021 across Europe.

Sexual dimorphism has been shown to influence vertebrate immunity through various factors, whose effects are mediated by epigenetic mechanisms, such as DNA methylation, non-coding RNAs, and histone modifications (Sasaki and Matsui, 2008). However, this relationship in fish remains underestimated and poorly studied (Caballero-Huertas et al., 2024). Our study uses cutting-edge techniques to investigate differences in genome-wide
DNA methylation patterns in the context of a sex-related immune response.

## Methodology

Samples from the gonads of European sea bass were collected 48 hours after bacterial infection with *Vibrio anguillarum*. Subsequently, genome-wide DNA methylation analysis was performed using the NEBNext® Enzymatic Methyl-seq Kit (EM-seq™) protocol with triplicate samples of each condition and gonadal tissue. Methylation patterns were assessed at each CpG site. Differentially methylated regions (DMRs) and differentially methylated loci (DMLs) were then identified for each comparison: between treatment conditions within the same sex (INF_F vs CTRL_F and INF_M vs CTRL_M), and between sexes under the same condition (CTRL_F vs CTRL_M and INF_F vs INF_M). After that, the different gene regions that overlapped with the DMRs and DMLs were found. Finally, these genes were annotated using the European sea bass genome as a reference, followed by functional enrichment analyses to identify associated Gene Ontology (GO) terms.

## Results

*Vibrio anguillarum* induced changes in the gonadal DNA methylation pattern across all comparisons. The highest number of differentially methylated CpG sites was observed when comparing sexes under the same condition, specifically in CTRL_F vs CTRL_M and INF_F vs INF_M, with 88,029 and 105,053 hypermethylated CpGs, and 2,829,573 and 2,752,371 hypomethylated CpGs, respectively. Our results suggested that there are more differences in DNA methylation between sexes under basal and infected conditions than after immune activation within the same gonadal tissue. Notably, the highest number of differentially hypermethylated CpGs was found after immune activation when comparing females to males, further highlighting the importance of sexual dimorphism in immune responses. When comparing the infected group against the control group within the same sex, in testes, we observed a higher number of differentially hypomethylated CpGs (53,489) than hypermethylated ones (50,099). In contrast, in ovaries, a higher number of differentially hypermethylated CpGs (94,329) compared to hypomethylated ones (68,876) was observed.

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## Discussion

These differences in the number of differentially methylated CpG sites suggest that sexual dimorphism plays a significant role in the methylation pattern of European sea bass, with females being hypomethylated compared to males under basal conditions. Furthermore, changes in the methylation pattern after the immune activation show a different tendency in each sex. Overall, in males, immune activation appears to induce a higher number of hypomethylated CpG sites compared to controls. In contrast, females show a tendency towards hypermethylation, which may be associated with gene repression. These findings highlight the importance of sex influencing the methylation status.

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## Acknowledgements

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# OXIDATIVE STATUS ACROSS THE ANNUAL CYCLE IN THREE CAPTIVE MANGROVE FISH SPECIES

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## Introduction

To assess fish welfare, physiological stress markers can be measured, with particular emphasis on those related to oxidative status. In this sense, glutathione metabolism plays a central role in cellular redox balance and defense against oxidative stress; however not all organisms respond to stress the same way, there may be differences between species from the same habitat. Moreover, lipid oxidative damage is a crucial indicator of animal welfare, reflecting cellular stress and health status. On the other hand, blood is the most commonly used sample, but to minimize alterations caused by stress from sampling, skin mucus is increasingly being used as an alternative. In addition, there are seasonal changes throughout the year, because of the circannual rhythms, that are to be considered. This study aims to seasonally characterize glutathione metabolism and lipid peroxidation levels in three mangrove teleost species held in captivity and to validate non-invasive sampling alongside oxidative status parameters as welfare markers.

## Materials and methods

*Scatophagus argus*, *Selenotoca multifasciata* and *Terapon jarbua* were maintained at the BioDomo of the Consorcio Parque de las Ciencias de Granada (Spain). Four samplings of mucus tegument of six fish of each fish species were conducted coinciding with seasonal changes: March (spring), June (summer), September (autumn), and December (winter). Six individuals of each species were sampled every season: mucus was collected. After processing, enzyme activities of glutathione peroxidase (GPX), glutathione S-transferase (GST) and glutathione reductase (GR), and levels of lipid oxidative damage (LPO) were determined. Protein concentration was determined by Bradford method. Results are presented as mean ± SEM. Two-way ANOVA was used, with species and season as fixed factors. As in all cases interaction among factors was detected, species and season effects were analyzed by a one-way ANOVA. F values were significant for $P<0.05$ and differences were determined following a post-hoc analysis using Tukey test. Statistical analyses were performed with SPSS v.25.

## Results and Discussion

Results for GPX, GST, GR, and LPO are shown in Figure 1. GPX activity peaked ($P<0.05$) in *T. jarbua* during spring and in *S. multifasciata* during summer, while GST activity was significantly higher in *S. argus* except in summer. Statistically, GR activity was highest in *S. multifasciata* during winter and summer, but in *T. jarbua* during autumn. LPO levels were generally highest in *T. jarbua*, except in spring, when *S. multifasciata* exhibited the greatest lipid peroxidation. No species consistently showed superior or inferior activities for enzymes related to glutathione metabolism, as patterns were season dependent; however, *T. jarbua* tended to display higher oxidative stress, as indicated by LPO values.

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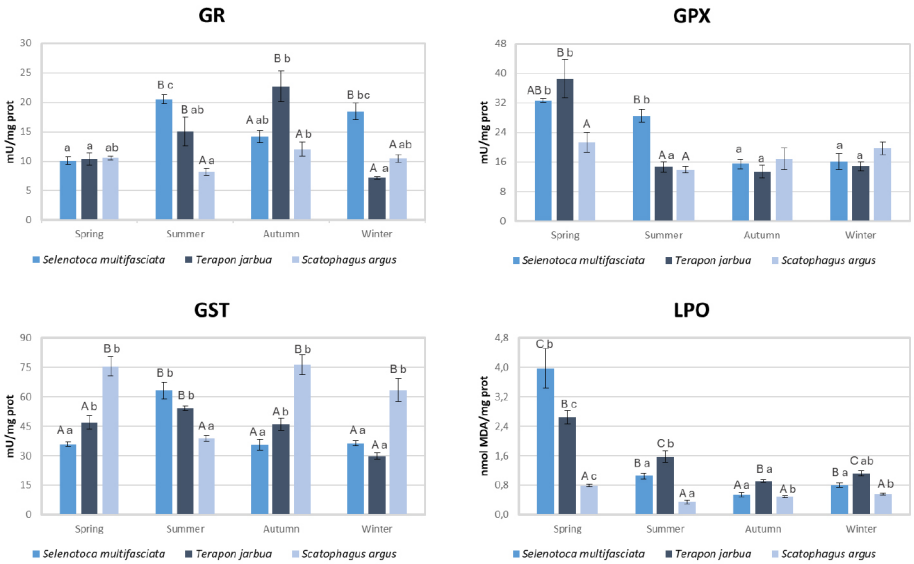


Fig.1. Specific activity for glutathione peroxidase (GPX), glutathione S-transferase (GST), glutathione reductase (GR) and levels of lipid peroxidation (LPO) in mucus of three mangrove fish species. Values are means ± SEM (n=6). Letters present statistical results from one-way ANOVA. Small letters indicate differences ($P<0.05$) among seasons for each fish species. Capital letters indicate differences ($P<0.05$) among fish species in each season.

On the other hand, seasonal trends revealed a circannual cycle for most parameters, with GPX, GST, and GR activities varying by species. So, GPX activity remained stable year-round in *S. argus* but significantly peaked in spring and summer for *T. jarbua* and *S. multifasciata*. For GST and GR, *S. multifasciata* reached maximum activities in summer, whereas *S. argus* recorded the lowest values in this season ($P<0.05$). However, lipid oxidative damage increased in spring across all species, suggesting higher stress during this period.

Overall, the data confirm species and season specific variation, with *T. jarbua* showing the highest stress levels. These oxidative stress parameters can serve as reliable welfare indicators and can be effectively assessed using non-invasive sampling methods.

**Acknowledgements**

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# PROFILING THE RAINBOW TROUT ERYTHROCYTE EPIGENOME IN FISH EXPOSED TO DIFFERENT IMMUNOLOGICAL STIMULI

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Recent studies in immunology have pointed out a process called trained immunity which is defined as the long-term functional reprogramming of cells of the innate immune system, induced by exogenous or endogenous challenges, leading to an enhanced response to a second challenge after a return to a non-activated state [1]. Trained immunity is known to be mediated by epigenetic reprogramming. In the immune system, epigenomic regulation has been observed during hematopoiesis, T cell memory, and B cell development. Additionally, epigenetic changes in monocytes and NK cells have been shown to imprint a form of innate immune memory [2]. Therefore, the concept of epigenetic imprinting on the immune system has gained particular relevance in the context of vaccination [3].

Fish red blood cells (RBCs) are gaining attention as a model for studying immune responses due to their active gene expression and participation in the innate and adaptive immune responses [4]. In the present work, we evaluated the epigenome of red blood cells (RBCs) from rainbow trout subjected to the viral hemorrhagic septicemia virus (VHSV), a DNA vaccine encoding the glycoprotein G of VHSV (pGVHSV), and an antiviral treatment based on green tea (*Camellia sinensis*). We used single cell Assay for Transposase-Accessible Chromatin sequencing (scATAC-seq), a high-throughput method used to identify open regions of chromatin across the genome, providing insight into the epigenetic landscape.

For this purpose, rainbow trout individuals were injected intramuscularly with the different stimuli mentioned above. After 3 days (or 5 days in the case of those individuals injected with pGVHSV), these individuals were sacrificed and RBCs were purified from blood from each individual. Then, we proceed with the characterization of chromatin accesibility by scATAC-seq.

The results demonstrated that vaccination and viral exposure showed distinct chromatin accessibility profiles, highlighting stimulated epigenomic remodeling of the immune response of RBCs, indicating activation of key regulatory elements.

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# IMPACTS OF DIFFERENT LAND-BASED PRODUCTION ENVIRONMENTS ON SKIN HEALTH IN ATLANTIC SALMON

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**Introduction**

Issues affecting skin health have been a persistent challenge in Norwegian Atlantic salmon (*Salmo salar*) aquaculture. The skin is a multifunctional organ, playing a crucial role in protecting against environmental stressors, pathogens, and physical injury. Beyond serving as a physical barrier, it also performs essential immunological functions, housing complex mucosal layers that contribute to immune surveillance, physiological homeostasis, and protection against harmful agents. As such, skin health is central to the overall robustness and performance of salmon in aquaculture.

The surrounding production environment closely influences the integrity and functionality of the skin barrier. Often regarded as a living sensor, the skin reflects the quality of rearing conditions, making it a key indicator of health in response to environmental factors. Before Atlantic salmon are transferred to sea, they are reared in various land-based systems, with recirculating aquaculture systems (RAS) becoming increasingly common. This shift has largely replaced the previously widespread use of flow-through systems (FTS) and partial-reuse systems (PRS). While RAS offers enhanced control over water quality, they are frequently associated with challenges such as elevated levels of ammonia, nitrite, and organic load, among other factors. These environmental parameters have been linked to compromised skin health, although direct comparisons between these systems remain limited.

This study investigates the effects of different land-based production environments on the skin mucosal barrier of Atlantic salmon, with a focus on immune function, oxidative stress responses, and overall skin physiology.

**Materials and Methods**

A total of 1800 Atlantic salmon parr, with an initial weight of 30-40 g, were reared under three different production regimes: a flow-through system (FTS), a recirculating aquaculture system (RAS) with 95% water recirculation, and a partial-reuse system (PRS) with 40% water reuse. During the first five weeks (Phase I), all fish underwent smoltification, with each system operating in freshwater at 12 °C. In Phase II (post-smolt stage), which lasted three weeks, the salinity in the FTS and PRS systems was increased to 33 ppt, and the water temperature was maintained at 8-10 °C. The RAS group was kept at the same temperature, but the system operated with 17 ppt brackish water. In the final phase (Phase III), all groups were transferred to flow-through systems with a salinity of 33 ppt and a temperature of 8-10 °C to simulate sea transfer conditions.

Skin samples were collected at the end of each phase for molecular and histological evaluations. Mucus was also collected and subjected to proteomic and metabolomic analyses. To simulate exposure to a secondary stressor, scale cultures from fish in Phase III were established and exposed to hydrogen peroxide ($H_2O_2$). Molecular and biochemical assays were then performed on these samples.

**Results and Discussion**

Preliminary histopathological analysis indicated an increase in the severity of pathological changes in the skin in phase III across all groups. Skin morphometric parameters, including epidermis thickness and the number of mucus cells present in the epidermis, did not reveal any significant differences between groups or phases. However, a significant increase in dermis thickness was observed after phase I in the FTS group.

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Gene expression analysis of skin samples from all production systems across the three phases showed a significant upregulation of immune- and stress-related genes in the PRS group during phase II. Mucin expression was significantly higher in the FTS group than in RAS and PRS groups, and other structural genes also showed a significant upregulation in the FTS and RAS groups in phase III. These findings suggest that production conditions influence skin functions related to barrier integrity and structure.

In the cell culture analysis, while the overall expression patterns of oxidative-stress genes were similar between control and stressed groups, there was a significant increase in the expression of some genes (e.g., *gpx*, *gr*, and *mnsod*) in the PRS group compared to the FTS group upon $H_2O_2$ exposure. In most of the oxidative-stress genes analysed, gene expression was lower in the FTS group than in the RAS and PRS groups, possibly indicating reduced cellular stress in salmon reared in flow-through conditions.

Oxidative stress biomarkers in the cell culture showed that in the FTS group, exposure to $H_2O_2$ led to an increase in total antioxidant capacity and a decrease in reactive oxygen species (ROS) and reactive nitrogen species (RNS), suggesting an efficient antioxidant system, characterized by a dynamic inverse relationship between antioxidant capacity and ROS/RNS levels. The opposite trend was observed in the PRS group.

Preliminary analyses of the mucus revealed differences in the protein and metabolite composition between the production systems, although data are still being analysed. These results will be presented during the meeting.

**Conclusions**

This study demonstrates that each land-based production system has a different impact on the skin barrier functions of Atlantic salmon, with a significant effect on immune and oxidative stress responses. Our findings suggest that the fish group reared in a partial-reuse system exhibited more changes in skin health conditions, exhibited by increased immune and stress responses, along with reduced antioxidant capacity, compared to fish from other rearing systems.

**Acknowledgements**

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# SEA URCHIN SETTLEMENT AND HABITAT SELECTION USING 3D-PRINTED SUBSTRATES

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## Introduction

The gonads of sea urchins are a gastronomic delicacy in several regions of the world, which has led to intense harvesting activity. As a result, the sea urchin fishery has become unsustainable. The purple sea urchin *Paracentrotus lividus* is a highly valuable species with a critical ecological role due to its wide distribution. For this and other sea urchin species, aquaculture provides alternative solutions to support wild population restoration as well as stock enhancement initiatives. Aquaculture for restocking purposes and the production of juveniles in controlled environments for further release in areas of over-exploitation helps boost populations and offers promising tools for resource management. However, the production of sea urchin juveniles is still laborious. The complex life cycle, with different stages of development associated with a high mortality rate, especially during the metamorphosis phase, constitutes a major challenge (Mendes et al., 2019). The present study aimed to control the metamorphosis phase and evaluate the feasibility of using 3D-printed clay substrates conditioned with different biofilms to enable the settlement process and improve the survival of juvenile sea urchins.

## Materials and Methods

Adult *P. lividus* urchins (4,20 ± 0,24 cm) were collected by hand on a rocky beach near Peniche and induced to spawn with an injection of potassium chloride. For fertilization, eggs and sperm from different animals were collected and mixed in a beaker in a ratio of 1 egg: 50 spermatocytes, guaranteeing genetic diversity and avoiding polyspermy. The larval rearing experiment was carried out in four 50 L cylinder conical tanks in closed system and maintained in a room with controlled temperature. Larvae were stocked at a density of 4 ind./mL, kept at a salinity of 35, and average temperature of 18 °C and reared in aerated static seawater. Larvae were fed with a mixed microalgal diet (*Chaetoceros calcitrans* and *Rhodomonas lens*) during their exponential growth phase. Larval competence was defined as the number of days post-fertilization (DPF) required for at least 75% of the larvae to reach competence for settlement (Gomes, 2019). To promote settlement, the larvae were transferred to 15 L aquariums containing 3D printed clay substrates conditioned with different biofilms: (a) a biofilm composed of two benthic diatoms, *Skeletonema costatum* and *Nitzschia acicularis*. cultivated in the laboratory - microalgae biofilm: (b) a biofilm composed of natural phytoplankton collected from a rocky beach and cultivated in the laboratory - indoor biofilm: (c) a biofilm naturally grown in the substrates emersed in Peniche harbour – outdoor biofilm. The substrates were conditioned with the different biofilm-forming conditions for eight weeks before sea urchin settlement. Just before the larvae attained competence, all the substrates were immersed in tanks holding adult sea urchins to acquire conspecific molecules. Then, the conditioned 3D-printed substrates were hung in the larvae rearing tanks to allow the competent larvae to metamorphose and settle on the surface of the substrate. Settlement success rate and sea urchin growth were assessed weekly.

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**Results**

The weekly evaluation of the settling rate between the different biofilm treatments revealed significant differences ($H_{(2)}$ = 23,122, *p-value* < 0,001). The microalgae biofilm treatment differs significantly from the indoor treatment ($Z_{(2)}$ = 24,021; *p-value* 0,005) and outdoor treatment ($Z_{(2)}$ = 40,698; *p-value* 0,001), There was a higher rate of settlement in the substrates conditioned with the outdoor biofilm, and the lowest settlement in the substrates conditioned with the microalgae. The weekly evaluation of the average growth of post-settled juveniles settled in the substrates revealed no significant differences in relation to the nature of the conditioning biofilm. Regardless, these results suggest a non-significant trend towards greater growth in the group settled in the substrates conditioned with the indoor biofilm.

**Discussion**

In marine benthic ecosystems, sea urchins have a substantial effect on the microenvironmental habiting the surface of temperate rocky shores. Juvenile sea urchins move using their spines and ambulatory feet and possess chemosensory abilities that allow them to detect nearby food sources (Solari et al., 2021) and select the most suitable habitats. This experiment demonstrates a preference of post-metamorphosed sea urchins for settling on surfaces conditioned with the outdoor biofilm. This result may have been influenced by biofilm´s closer resemblance to natural conditions. In contrast, the microalgae-based biofilm showed lower settlement success. This may be due to the biofilm not being fully developed, despite the benthic diatom used to form the microalgae biofilm being commonly found in rocky areas covered with macroalgae mats. In future studies, the substrates should be conditioned for a longer period in areas where the natural diet of these organisms is more readily available.

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# DAILY EXPRESSION OF CLOCK GENES AND LIPID METABOLISM-RELATED GENES IN *Epinephelus malabaricus* AND THE EFFECTS OF RESTRICTED FEEDING

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Biological clock regulates daily rhythms in behavior, physiology, and metabolism. The central clock in the brain is synchronized by the photoperiod, whereas peripheral clocks in peripheral tissues such as the liver may be influenced by other synchronizing factors. This study aimed to elucidate the role of clock genes in the lipid metabolism of the Malabar grouper *Epinephelus malabaricus*. To achieve this, we examined the diurnal expression patterns of clock genes in both diencephalon (central tissues) and liver (peripheral tissues), along with the diurnal expression of genes involved in the liver lipid metabolism.

Juvenile Malabar groupers used in this study were acclimated in a flow-through seawater tank under natural photoperiod conditions (sunrise at 0630 h and sunset at 1800 h). Following acclimation for two weeks, fish (n = 5 per time point) were sampled 4-hour intervals beginning at 0700 h. The diencephalon and liver were collected after anesthesia. RNA was extracted from both tissues, reverse-transcribed into cDNA, and analyzed by the real-time polymerase chain reaction to measure the expression levels of clock genes and lipid metabolism-related genes. Clock genes included brain and muscle ARNT-like (*bmal1* and *bmal2*), period circadian regulator 2 (*per2*), cryptochrome circadian regulator 2 (*cry2*), and circadian locomotor output cycles kaput (*clock*).

Clock genes (*bmal1*, *bmal2*, *cry2*, and *clock*) in the diencephalon and liver peaked during the photophase. In contrast, lipid metabolism-related genes (*cpt1, fas, srebf1*) exhibited daily expression patterns with lower expressions during the photophase and higher expressions during the scotophase. Many of these genes were associated with lipolysis. These results suggest that the expression of lipid metabolism-related genes may be influenced by clock genes expressed in either the central or peripheral tissues.

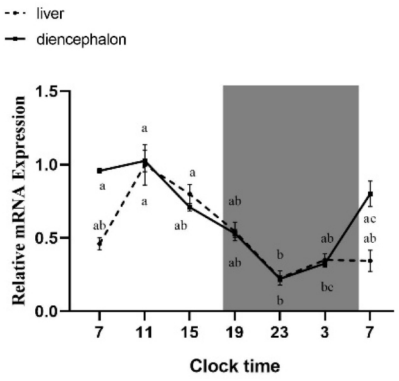


Fig. 1. Daily changes in the transcript levels of clock gene (per2) in the diencephalon and liver of the Malabar grouper. Significant differences between time points are indicated by different lower-case letters. Gray background represents dark period.

# OPERATIONAL MONITORING AT AN AQUACULTURE FACILITY

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**Introduction:**
The advancement of offshore aquaculture has necessitated the development of robust and resilient infrastructures capable of enduring variable and harsh marine environments. Nonetheless, significant concerns persist regarding the efficacy of current conventional system designs, monitoring protocols, and maintenance strategies, primarily due to frequent mooring system failures and structural deformations induced by the synergistic effects of hydrodynamic forces such as currents, wave action, and wind loads.

The AQUAMOORE project, funded by INFO Región de Murcia, seeks to address these challenges through the engineering and deployment of an advanced sensor network integrated with a data analytics framework on a mussel longline platform. This system facilitates continuous real-time monitoring of both structural integrity and environmental parameters, enabling early detection of potential failure modes and risk scenarios. Moreover, it supports the implementation of predictive maintenance strategies grounded in numerical simulations, alongside growth modeling to optimize mussel production.

**Materials and methods:**
A load sensor system with satellite data transmission was installed for continuous monitoring of tensions in production ropes, alongside a current meter to assess sea state conditions.

The working methodology comprised the development of an analytical tool, the execution of seakeeping simulations, and the creation of a machine learning model and digital twin for tension prediction. All results were compared and validated against data collected by the sensor system on two selected days representing storm and calm sea conditions.

This process was initially developed through a simple pilot based on the mooring of a buoy to validate the methodology by studying the correlation between models. Subsequently, the described methodology was applied in a pilot project involving AZTI's mussel raft located in Mutriku (Basque Country, Spain) (Figure 1), aimed at determining tension in three production ropes and assessing the effect of current on these parameters, thereby enabling estimation of biomass at any given time. Two of the ropes were loaded with known deadweights, while the third carried mussels.

**Results:**
The results obtained from the various tools in the buoy pilot validated the implemented methodology. The average stress values (mean tension) from the time series collected by the sensors and numerical simulations were comparable and of the same order of magnitude, with errors below 13%. Additionally, the machine learning model showed a correlation coefficient of $R^2 = 0.834$ relative to the tension measured by the sensors.

Regarding the mussel raft pilot, the conducted study enabled precise characterization of the stresses experienced by three installed production lines. Through analytical, numerical, and experimental methodologies, simulation models were successfully validated against real sensor data. The analytical tool proved effective in estimating tensions under moderate current conditions. However, the machine learning model did not achieve a valid correlation, as current was the sole input variable, which proved insufficiently representative at the raft's location.

The sensor data indicate a progressive increase in load on the mussel line, reflecting the expected biomass growth. In contrast, the lines equipped with deadweights exhibited lower and more consistent load values, potentially attributed to the accumulation of biofouling over time.

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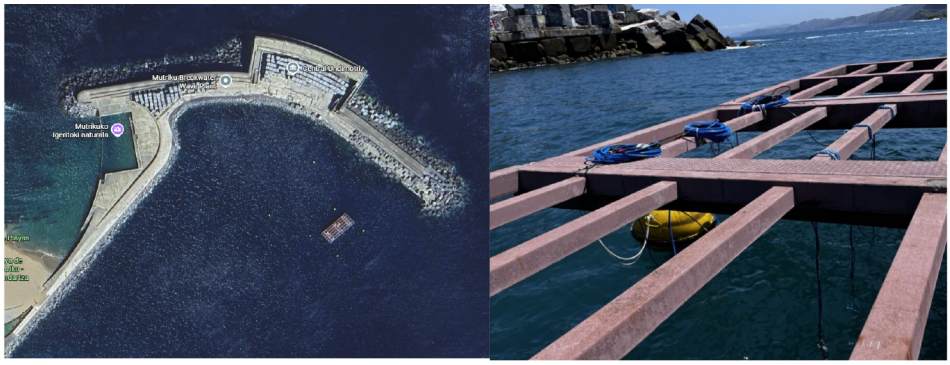

*Figure 1. Mutriku raft and installed sensor system.*

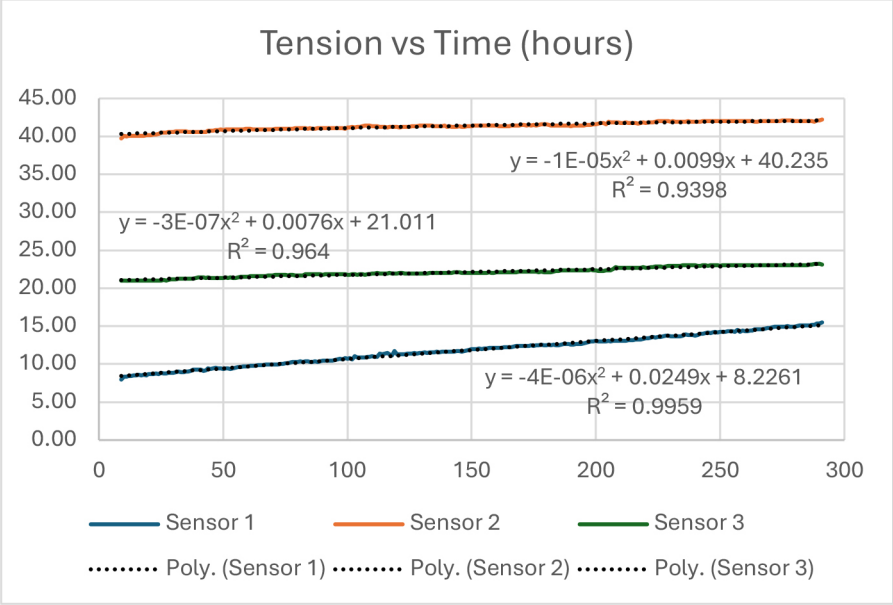


*Figure 2. Pilot raft results*

**Conclusion and future work:**

The results from the buoy pilot validated the working methodology proposed in the project.

The data collected from the mussel raft demonstrated the feasibility of measuring the tension exerted on the production line. Furthermore, the equations derived for each line confirmed the validity of the system, as the extrapolated values at X = 0 closely matched the initial wet weight of each line. The analytical tools and numerical simulations yielded satisfactory results, whereas the machine learning model did not achieve a strong correlation, primarily due to the minimal current observed in the raft's environment, and therefore its lack of representativeness, being the sole variable of the model.

Subsequently, the tension measurement system for weight estimation will be deployed on a longline, where current is expected to have a greater influence on the production lines and additional variables, such as wave loads, will also be monitored.

This new deployment aims to decouple the contribution of current to the measured tension in each line and to estimate the biofouling accumulation rate. Ultimately, this will enable accurate estimation of the biomass present on each line. The resulting data will be compared with a study conducted by AZTI in 2024 [1] on mussel growth in this type of production system.

**Reference**

[1] Arantzamendi et al, 2024, July. Assessing the mechanical properties of biobased versus fossil-based ropes and their impact on the productivity and quality of mussel (Mytilus galloprovincialis) in longline aquaculture: Toward decarbonization

# RAISING THE BAR ON AQUAFEED SUSTAINABILITY: TRANSPARENCY, EMISSIONS, AND SOCIAL DUE DILIGENCE

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Identifying and addressing climate and social risks in aquaculture calls for a holistic, value chain-wide perspective. The Aquaculture Stewardship Council (ASC) supports this through its robust Farm and Species Standards, which establish best-practice requirements for producers to strengthen environmental and social performance at the farm level. In addition, the ASC Feed Standard extends this responsibility upstream, targeting the significant impacts of feed production, one of the largest contributors to aquaculture's overall footprint. These impacts include greenhouse gas (GHG) emissions, habitat conversion, resource depletion, and labour and human rights risks, many of which arise during the sourcing of feed ingredients, particularly from agriculture associated with land-use change. As climate pressures intensify and competition for land and resources grows, the need for transparent, sustainable, and socially responsible feed systems is more urgent than ever.

ASC's Feed Standard addresses these challenges through a robust, supply chain-wide framework. It sets clear environmental and social requirements for feed mills and their suppliers, including the protection of high conservation value ecosystems, responsible ingredient sourcing, and improved feed efficiency to lower emissions. Socially, the Standard embeds international labour and human rights expectations, requiring due diligence on issues such as child labour, forced labour, and working conditions. It also enhances supply chain transparency through risk-based procurement and traceability requirements.

A key enabler of this transition is the ASC GHG Calculator, a practical tool that allows feed mills and producers to quantify, compare, and report emissions in a consistent and credible way. It helps identify emission hotspots, and combined with the Feed Standard, helps supporting mitigation planning, and facilitating alignment with evolving climate and due diligence regulations. By discouraging the use of high-risk ingredients and enabling informed decision-making, the ASC helps in advancing climate-smart and socially responsible aquaculture.

This presentation explores the rationale, design, and implementation of the ASC Feed Standard and GHG Calculator, and their potential to drive measurable improvements in environmental and social performance across the aquaculture feed sector.

# GUARANTEED BONE FREE? NOVEL INTERMUSCULAR BONE ABNORMALITIES IN CHINOOK SALMON *Oncorhynchus tshawytscha* AND THEIR IMPLICATIONS FOR AQUACULTURE

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## Introduction

Skeletal deformities are a persistent welfare and production issue for salmonid aquaculture, including New Zealand farmed Chinook salmon (*Oncorhynchus tshawytscha*). Research on deformities of the fine bones, such as intermuscular bones (IBs) and ribs, have received much less attention than vertebral deformities in salmon musculoskeletal research[1-3]. IBs, commonly known as pin bones, are small spicule-like bones, present in the muscle fillets of basal teleost species. IBs are removed during fish processing, as they present a human health risk if ingested. IB abnormalities can cause technical difficulties in the automated or manual removal of IBs at processing. Furthermore, the proposed role of IBs in swimming efficiency, emphasises the potential welfare implications of IB abnormalities. However, there are significant knowledge gaps about the function and development of IBs[1]. IB abnormalities were first identified on a significant scale in a population of harvest-size Chinook salmon, during fish processing. The IBs presented as incompletely mineralised at the proximal end, which caused difficulties in removal[1] (Fig. 1). To our knowledge, this is the first account of IB abnormalities in farmed salmonids in the literature. Thus, we aimed to characterise this novel bone abnormality and identify any association with vertebral deformities.

## Materials and methods

A subsample of the affected population (n = 118), (weight = 5081.86 ± 134.69 g, fork length = 679.07 ± 5.5 mm, mean ± SEM) previously stored at -20°C, were weighed, measured, and whole-body and fillets x-rayed. The prevalence and severity of IB abnormalities and vertebral deformities were scored from x-rays. Fish were categorised into two groups: <25% abnormal and >25% abnormal IBs. Normal and abnormal bones (n = 71 normal and 61 abnormal bones from 16 fish, n = 8 fish per group) were selected for: mechanical (breaking load) analyses using a TA.XT texture analyser; calcium and phosphorus content via ICP-MS (n = 12 fish); and osteocyte density by histological examination (n = 6 fish). The significance level was set at $p \leq 0.05$ for Wilcoxon signed-rank tests. For analyses conducted within the Bayesian framework, effects were considered meaningful if the 95% credible interval (CI) of the posterior distribution excluded zero.

## Results and discussion

The individuals assessed had 62 ± 0.18 IBs (31 per fillet). IB abnormalities were present in all fish sampled, with prevalence varying among individuals. Fish with higher prevalence, (>50% of abnormal IBs), were smaller (4773 ± 126 g) than those with lower prevalence (<10%) (5464 ± 178 g). The position of abnormal IBs along the vertebral column was asymmetric. The left fillet had a significantly higher ($p$ <0.001) number of abnormal IBs than the right fillet (9.85 ± 0.58 vs 7.82 ± 0.51). Vertebral deformities were also present in over half of the fish, i.e. compression, fusions or vertical shifts (25%), spinal curvature (14%) or both (15%). There was no association between IB abnormalities and vertebral deformities occurrence (β = -0.06, 95% CI = -0.31, 0.18).

Abnormal IBs had significantly lower (β = -3.43, 95% CI = -5.56, -1.32) breaking load, but only a numerically lower phosphorus content ($p$ = 0.72). IBs of fish with >25% abnormal IBs had significantly lower (β = -2.34, 95% CI = -4.12, -0.51) breaking load for both normal and abnormal bones, relative to the <25% group. No difference in mineral content between normal and abnormal bones suggested the need to compare unaffected fish.

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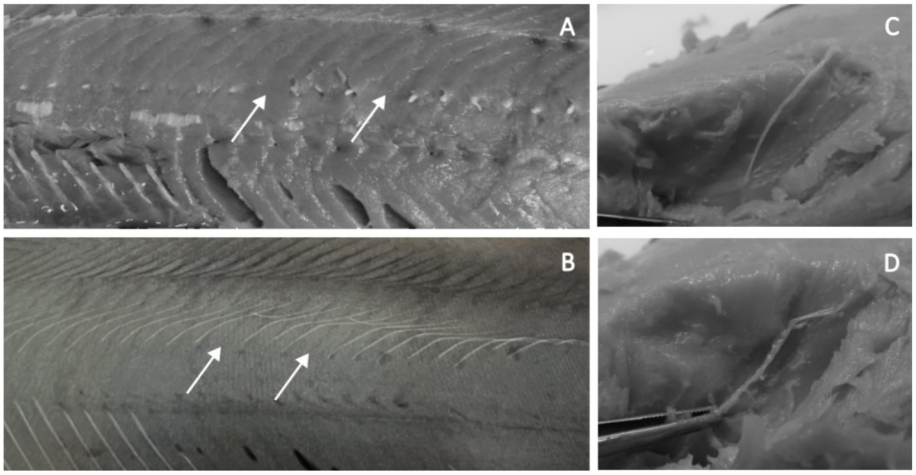


**Fig. 1:** Photograph (A) and radiograph (B) of a harvest-size Chinook salmon fillet, highlighting the missing/radiolucent proximal ends of the abnormal IBs (white arrows). C) Normal IB with a bony proximal end above the fillet surface. D) abnormal IB with a proximal end of non-mineralised collagen matrix, below the fillet surface.

Abnormal IBs had significantly fewer osteocytes than normal bones (preliminary analysis). The reduced number of osteocytes in abnormal IBs may be indicative of variation in bone growth rate and mineralisation. Our findings suggest that the physical and mechanical changes in abnormal IBs are indicative of altered IB development, and possible bone remodelling issues. Moreover, IB abnormalities are independent of vertebral deformities in Chinook salmon. The occurrence of IB abnormalities is a unique example of how musculoskeletal disorders can go by undetected, with unknown consequences on welfare and performance, and until fish are on the plate and it's too late.

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# APPARENT DIGESTIBILITY OF A BACTERIAL SINGLE-CELL PROTEIN IN JUVENILE ATLANTIC SALMON *Salmo salar* AND THE PRELIMINARY EFFECT ON PALATABILITY

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## Introduction

The rapidly growing aquaculture sector is recognized as critical to supplying global protein needs, and protein-rich ingredients are required to fill the protein gap in feed production. Single cell proteins (SCPs) such as microalgae, yeast or bacteria are novel ingredients that have all been tested as alternative protein sources for aqua feeds (Glencross et al., 2020). Among the SCPs, bacterial SCPs are some of the most popular and sustainable ingredients, with a high protein content and an amino acid (AA) profile resembling that of fishmeal (Pereira et al., 2022). At the same time, SCPs derived from microorganisms grown on gas offers a sustainable high-quality protein, which can be intensively produced without having a negative impact on the environment (Glencross et al., 2020). The objective of the present study was to assess the digestibility of a bacterial SCP as a viable alternative protein source in the diets of Atlantic salmon (*Salmo Salar*).

## Materials and methods

The diets were formulated based on a 70:30 digestibility model, with the test ingredient, Proton (crude protein = 87.6%), composing 30% of the experimental diet. The control feed was formulated to resemble a commercially available salmon feed for parr to pre-smolt stage. The experimental diets were fed to Atlantic salmon with initial mean weight of 76.18 ± 4.61 g for 49 days in a freshwater recirculating aquaculture system. Before starting the digestibility trial, a random sample of 20 fish were subjected to gross pathology health checks, to ensure a healthy population for the trial. Fish were acclimated to the trial system and fed a commercial diet for 10 days, after which the fish were acclimated to the experimental diet for 6 days, before digestibility collections were initiated. Fish were fed to apparent satiation across three meals per day, and after each feeding round the tanks were cleared for any material (uneaten pellets or faeces). After 15 minutes, the collection of faeces commenced, and continued periodically until no further material was present, or the next feeding round began.

All data was analysed with RStudio, and apparent digestibility coefficient (ADC) was calculated based on the following equation (Zehra et al, 2024):

$$ADCing = \frac{(ADtest \times Nutrtest - (ADbasal \times Nutrbasal \times 0.7))}{(0.3 \times Nutringredient)}$$

Where *ADCing* is the digestibility of the nutrient from the test ingredient (Proton), *ADtest* is the apparent digestibility of the nutrient from the test diet (30% Proton inclusion), *ADbasal* is the apparent digestibility of the nutrient from the basal diet (Control) which makes up 70% of the test diet, and *Nutringredient*, *Nutrtest* and *Nutrbasal* are the levels of the nutrient in the ingredient, test diet and basal diet, respectively.

## Results

There was a statistically significant higher average individual weight gain of the salmon ($p = 0.018$) in the experimental group compared with the control group after 49 days of feeding (53.90 ± 2.72 % and. 45.75 ± 5.95 %, respectively). Additionally, the experimental group had a higher feed intake (2.43 g/day per fish vs. 2.34 g/day per fish) and significantly less faecal matter per tank ($p = 0.015$) compared with the control group (9.55 ± 0.59 g and 12.52 ± 1.73 g DM, respectively). Pairwise comparison of ADC between the diets demonstrated that the average ADC values for protein, GE and AAs were statistically higher for the experimental diet compared to the control diet ($p < 0.003$). Furthermore, Proton demonstrated a protein ADC of 94.1% and average amino acid ADC of 95.6%.

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**Discussion and conclusion**

Digestibility is a critical factor when evaluating novel protein sources for aquafeeds, particular in juvenile Atlantic salmon that require a high-quality protein for optimal growth. The high protein ADC from Proton demonstrated in this trial suggests a superior nutrient utilisation compared to many alternative protein sources tested in salmonid diets (Øverland et al., 2013; Gong et al., 2018). The high protein ADC is even comparable to other high-quality proteins like fishmeal with ADC values ranging from 80-90% (Øverland et al., 2009). At the same time fishmeal typically provides high digestibility coefficients of essential AAs exceeding 90% (Storebakken et al., 1998), which Proton in the present study could compete with at an average AA digestibility of 95.6%.

Over the duration of the trial, fish receiving the experimental diet had a slightly higher feed intake compared with the control group, which may indicate an improved palatability even at high inclusion levels of Proton. On the other hand, differences in nutrient composition due to the unbalanced diets, must be considered when interpreting the data. The experimental diet had a higher protein content due to a 30% inclusion of Proton, which resulted in not fully reliable growth data. However, the growth results give an indication of how Proton might perform in a dedicated growth trial, and it supports the high digestibility claim. The study indicates that Proton is a highly digestible alternative protein ingredient for juvenile Atlantic salmon, with strong ADC values for protein and AA. The preliminary performance also demonstrates a good palatability of a Proton diet. The findings highlight Proton's potential as a sustainable and effective protein source in salmonid diets.

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# BACTERIAL SINGLE-CELL PROTEIN AND ITS HYDROLYSATES AS A PERSPECTIVE FEED ADDITIVES FOR FISH ARTIFICIAL DIETS: A CASE OF PIKE-PERCH (*Sander lucioperca*)

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## Introduction

The development of industrial pike-perch rearing technology has been limited by the lack of larval artificial diets that can fully meet the nutritional requirements of fish in the early stages of ontogeny. Previous attempts to grow pike-perch on a mono-diet of artificial feed from the start of exogenous feeding have failed. Total larval mortality occurred during the first 2-3 weeks of feeding. To solve this problem, we studied the proximate composition of the natural diet of pike-perch larvae, which consisted mainly of zooplanktonic organisms. We assumed that it should reflect the nutritional requirements of early larvae (Lyutikov et al., 2025).

The obtained results of the proximate compositional analysis of zooplankton allowed us to select the most suitable feed component for the development of artificial starter feed for pike-perch larvae, which was a microbiosynthetic protein - Bacterial Single-Cell Protein (BSCP) based on methane-oxidising bacteria *Methylococcus capsulatus*. BSCP contains similar protein and nucleic acid levels, amino acid composition and molecular weight distribution of peptides as zooplanktonic organisms. The aim of this study was to evaluate the efficacy of BSCP in both intact and hydrolysed forms as part of the initial diet of pike-perch larvae.

## Materials and Methods

The studies were conducted at the "Forvat" LLC fish farm (60°36'14.91"N 30°23'53.54"E). The larvae were obtained from natural spawning of 4-year-old pike-perch, reared from yearlings under industrial conditions in cages on artificial diets only. After becoming horizontal, the larvae were placed in production square tanks (in 3 replicates) with an area of 4 $m^2$ and a water level of 0.25 m. The stocking density of the larvae was 25 individuals $L^{-1}$. The larvae were fed hourly from 8 a.m. to 10 p.m. The photoperiod, lighting and water temperature were natural throughout the experiment; the water temperature increased steadily from 16 to 21°C during the experiment. BSCP ("GIPROBIOSINTEZ" LLC, Russia) and its hydrolysates were added to the diet at the stage of mixing of the diet components, which was then extruded. The extrudate was dried, crushed and dispersed into 0.1 to 0.3 mm fractions. A mixture of fish oil and phospholipids was added to the diet, dried and packed in vacuum bags, which were stored at -18 °C until use.

## Results

After 23 days of rearing, the body weight, SGR and surviving of pike-perch larvae were highest in the group of fish fed diets containing BSCP L (low degree of hydrolysis). The number of non-feeding larvae in this group was less than 1% (Table). Using BSCP H in diets (high degree of hydrolysis) resulted in a decrease in body weight, growth rate, and surviving of pike-perch larvae (Table). The proportion of non-feeding larvae in this group was 57%. The larvae fed with diets containing native BSCP showed intermediate values of estimated characteristics (Table).

Table. Pike-perch larvae fed by diets with BSCP and its hydrolysates. The initial body weight of the larvae was 0.4 mg. Values (mean±SD) in the same row with lower case letters (a, b, c) are significantly different at $P \leq 0.05$.

| Diet | Body weight, mg | SGR, % | Surviving, % | Non feeding larvae, % |
|---|---|---|---|---|
| BSCP | 14,6±0,6$^a$ | 15,6±0,6$^a$ | 12,2±2,2$^a$ | 15,0±2,6 |
| BSCP L | 23,2±0,8$^b$ | 17,6±0,7$^b$ | 25,4±6,1$^b$ | ≤ 1,0 |
| BSCP H | 9,1±0,7$^c$ | 13,5±0,8$^c$ | 5,9±1,4$^c$ | 57,0±4,8 |

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It is clear that BSCP L has a positive effect on the growth, survival and feeding of pike-perch larvae, due to an increase in the amount of low-molecular-weight peptides (compared to intact BSCP) available for absorption by larvae whose digestive system is immature. On the other hand, BSCP H leads to a sharp increase in the proportion of peptides and amino acids, which has a negative effect on the fish rearing.

**Conclusion**

Obviously, the main direction of industrial farming technology of larvae and juvenile pike-perch will be the development of complete artificial diets capable of satisfying the nutritional needs of fish. For this, the BSCP and its hydrolysates are suitable sources with the necessary amount of peptides and free amino acids, which is especially important during the period of rapid growth of pike-perch.

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# CEJKO SOLUTION AND CHILLED CONDITIONS PRESERVE CARP SPERM QUALITY AND FERTILIZATION PERFORMANCE DURING SHORT-TERM STORAGE

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Short-term storage of fish milt is a cost-effective strategy in aquaculture as it enables synchronization of sperm and eggs. However, the optimal handling conditions immediately after milt collection remain unclear. In this study, we evaluated four storage conditions (diluted 1:1 with Cejko solution, undiluted, on ice, and at room temperature) over a 6-hour period in carp (*Cyprinus carpio L.*). Cejko solution–diluted groups exhibited significantly higher sperm kinetics and fertilization ability compared with undiluted groups. Sperm motility, curvilinear velocity (VCL), and straight-line velocity (VSL) decreased significantly during storage in both undiluted groups, with the room-temperature (RT) group showing lower motility than the on-ice (OI) group. Fertilization and hatching rates did not differ significantly between diluted-on-ice and diluted-room-temperature groups but declined markedly in both undiluted groups. These findings indicate that carp sperm maintain the best quality and fertilization capacity when diluted with Cejko solution and stored on ice, and dilution should occur within 6 hours of collection.

## Introduction

Short-term in *vitro* storage of sperm has become a common technique used to support and optimize artificial reproduction in aquaculture (Cejko et al. 2022; Contreras et al. 2017; Shazada et al. 2025). Sperm immediately diluted with Cejko solution show no significant differences in motility, fertilization and hatching rate compared with sperm diluted after 3 hours in carp (Shazada et al. 2025). However, there is currently no reference time threshold to guide fish farmers in performing short-term sperm storage. Raising the temperature to 20 °C before fertilization has been shown to improve sperm kinetic parameters and fertilization potential (Zhang et al. 2023). All current studies have directly used chilled temperature to perform sperm short-term storage in carp (Saad, et al. 1988; Cejko et al. 2019; Dietrich et al. 2021). Nonetheless, the effects of room temperature, which is more convenient for hatchery practice, have not been evaluated.

## Materials and methods

Mature males of carp were obtained following fish farm pond harvesting and maintained in a laboratory and hatchery aquatic system. Sperm and eggs samples were collected individually after treatment with carp pituitary extract at 21°C. Sperm was diluted with carp extender (Cejko et al., 2018) and stored for 6 hours under four different conditions, followed by assessment of spermatozoa motility, velocity and hatchability (Fig. 1).

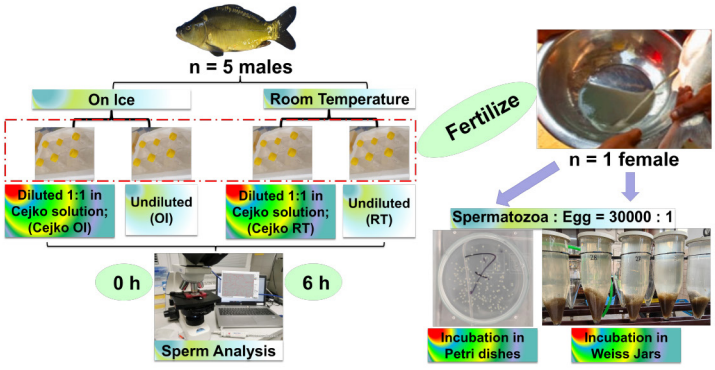


Fig. 1 Schematic figures illustrate the experimental design of the study.

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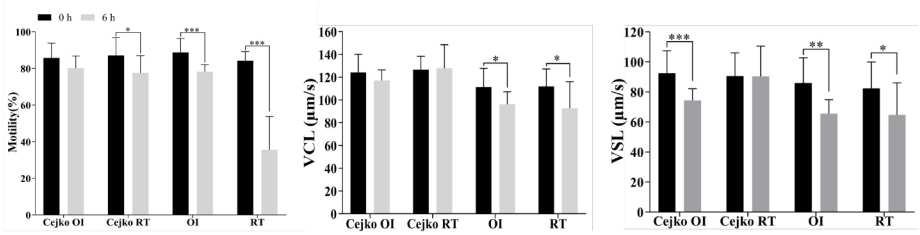


Fig. 2 Sperm kinetic parameters declined in undiluted conditions

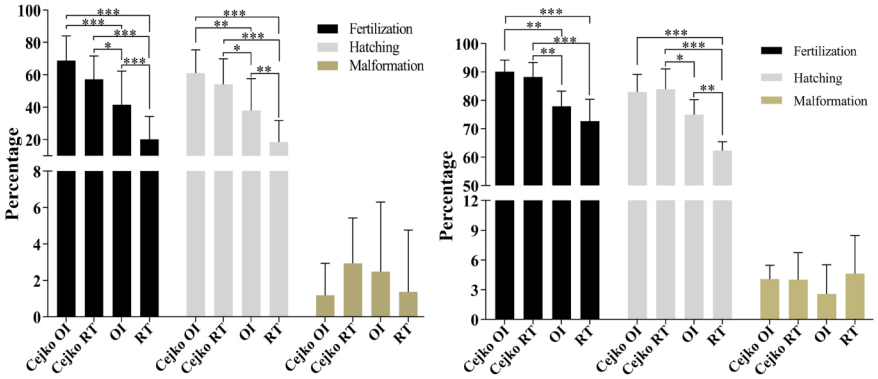


Fig. 4 Cejko solution and stored on ice preserve carp sperm fertilization ability

**Results**

The kinetic parameters of undiluted carp sperm (motility, curvilinear velocity [VCL], and straight-line velocity [VSL]) declined significantly during 6-hour storage, with sperm motility in the room-temperature (RT) group being significantly lower than in the on-ice (OI) group. Sperm diluted 1:1 with Cejko solution and stored on ice (Cejko OI) showed no changes in motility or VCL over 6 hours. Although sperm motility declined significantly in samples diluted 1:1 with Cejko solution and stored at room temperature (Cejko RT), both VCL and VSL remained stable (Fig. 2).

No differences in fertilization or hatching rates were observed between the Cejko OI and Cejko RT groups in either the Petri dish experiment or the Weiss jar experiment. Fertilization and hatching rates in the undiluted groups (OI and RT) were significantly lower than those in the diluted groups (Cejko OI and Cejko RT). Moreover, room temperature significantly reduced fertilization and hatching rates in undiluted samples (RT), whereas no such differences were detected in diluted samples (Cejko RT).

**Conclusion**

- ► Dilution at a 1:1 ratio with Cejko solution and storage on ice is optimal for short-term storage of carp milt in vitro.
- ► Carp sperm should be diluted within 6 hours after collection to maintain quality.

**Funding information**

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# TECHNOLOGICAL FRAMEWORK FOR DELIVERING MICROBIAL MODULATORS TO RAS

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## Introduction

Aquaculture systems are critical for global food security. Current cultivation techniques often disrupt the equilibrium of microbial communities, compromising system resilience and harming vital ecosystem services provided by microbes. Some interest in microbial interventions has emerged in the last decade. However, strategies remain focused on the host, the majority of which centred around the gut microbiome. Despite the increasingly obvious benefits of engineering the gut microbiome, the industry and scientific community is overlooking a system-wide strategy, as well as the interactions between fish, water and biofilm communities. The variety of micro-habitats and rich microbial diversity naturally present in aquaculture systems has the potential to improve productivity, health, sustainability and the stability of the system.

## Materials and Methods

This work explores the role of microbial modulators (humic substances [HS] and *Eisenia bicyclis* biomass [EB], and their combination [MIX]) as tools to shape microbial communities across key aquaculture biotopes (water, tank wall biofilms, and fish skin mucus) of a pilot-scale recirculating aquaculture system (RAS), using European seabass (*Dicentrarchus labrax*) as a model organism. To deliver the microbial modulators, this work uses a patent-pending framework that takes advantage of the biodegradable properties of polycaprolactone to release the microbial modulators in a controlled manner.

## Results

Results show an effective modulation of microbial communities across the studied biotopes. Interestingly, community changes were specific both to biotopes and modulators, including the increase of putative beneficial bacteria. As an illustration of how microbial communities have the ability to provide important services to the RAS, nitrification was accelerated by HS, delayed by EB, and all treatments except the control showed a reduced total nitrogen content. These results were possible without adversely affecting water quality, fish health or performance.

## Conclusion

This proof-of-concept provides foundational evidence for an effective early-stage technology that can be used as a complement or alternative to conventional water treatment methods, which has the potential to improve water quality, pathogen resistance and system resilience.

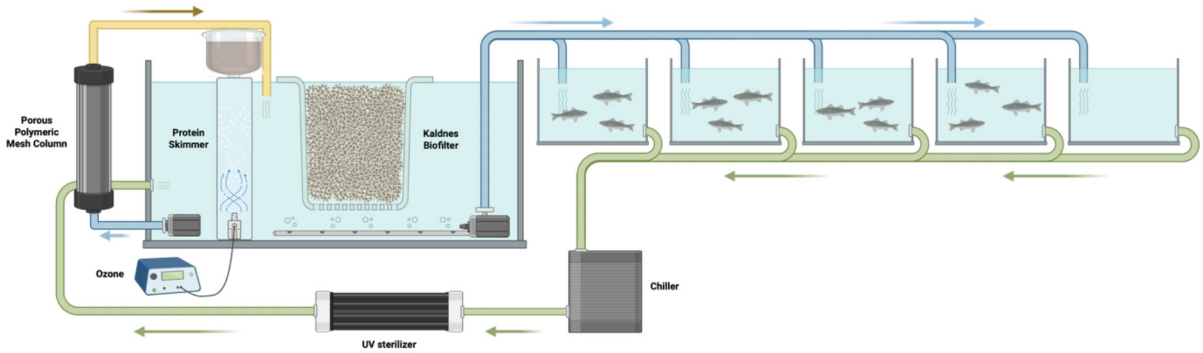


*Figure 1- General representation of the RAS.*

# aquaculturR: A COMPREHENSIVE R TOOL FOR ZOOTECHNICAL METRICS

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## Background and relevance

Aquaculture research has grown rapidly over the last decades, mirroring the global trend of aquaculture production and demand. The growing complexity of aquaculture research and production, demands more robust data analysis and modelling tools to project, maintain and assess stocks in aquaculture practices. To calculate growth performance and the health status of cultured species, a wide range of zootechnical parameters are routinely used as key performance indicators.

Examples for common key aquaculture performance indicators are, for example, the Feed Conversion Ratio (FCR), Nutrient Use Efficiency (NUE), Specific Growth Rate (SGR), Thermal Growth Coefficient (TGC), and Apparent Digestibility Coefficients (ADC). Interestingly, discussions have repeatedly flared up about how these seemingly trivial indicators should be calculated correctly. For instance, non-standardized metrics, approximations, inconsistencies, and outdated or inadequate references are highly prevalent in aquaculture studies, which resulted in several scientific publications devoted to discussing these metrics, *e.g*. SGR (Crane et al., 2019), FCR (Glencross et al., 2024), or ADC (Bureau and Hua, 2006). However, scientific discussions do not help those who do not have access to the publications in question, nor do they help scientists who are still developing a broad overview of the subject matter. Additionally, the lack of uniformity undermines the quality of the data and harms replicability, reproducibility and cross-study comparability. The increasingly ubiquitous use of artificial intelligence (AI) could further exacerbate the problem. Since large language models predict their responses based on the probability that a certain word or equation follows on the prior output, they might not distinguish between different equations for a metric but rather use the one that occurs more frequently in the underlying data set.

To address this gap, we introduce an open-source R package aiming to standardize and centralize the most commonly used zootechnical equations in aquaculture studies. The package offers a comprehensive bundle that facilitates accurate and consistent calculations, which is easily accessible to students, early career researchers and experts.

Table 1: Metadata of the aquaculturR package for R.

| | |
|---|---|
| Current code version | v0.3.0 |
| Link to code repository | https://github.com/TellAnAx/aquaculturR |
| Legal code license | GPL-3.0 |
| Code versioning system | Git |

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## Software description and metadata

"aquacultuR" is an R package that provides functions to compute common indices such as SGR, TGC, and ADC, among others. The use of GitHub as a platform allows for reporting bugs, suggesting changes, and contributing to the existing codebase. Additionally, it can serve both as a discussion hub and knowledge base to support the development of the package and underlying equations. Meanwhile, R as software is open source, free of charge and thus widely accessible for researchers and aquaculturists around the world. In addition, the code can easily be translated into an interactive Shiny app, allowing for user-friendly graphical interfaces.

The "aquacultuR" package implements common indices using standardized objects and functions. The code is based on best practices, following the standards for R software development (Wickham, 2023). Each function is tested for data inconsistencies that may impact or invalidate data analysis. In addition to the standard documentation, the code includes a vignette that elaborates on all functionalities in greater detail, with up-to-date references, and a formulary for quick and easy access to all the main performance indices, and common examples. Version control and an accessible collaboration environment have been ensured by hosting the code repository on GitHub.

## Impact

Currently, AI is an effective tool to conduct data analysis. Meanwhile, the quality of the outcomes depends on the quality of the training data. With widespread adoption, the "aquacultuR" package will contribute to injecting R functionalities of peer-reviewed quality into databases. Well-documented metrics, alongside most relevant literature and formalized equations will provide, for the first time, a comprehensive and collaborative overview that could help with learning and clarifying how common calculations are correctly made and highlight differences between existing equations, while simultaneously making those metrics more accessible for researchers. Additionally, the package offers a citable reference for calculations in scientific publications, making it unnecessary to state equations unless they have been specifically modified, streamlining writing and peer-reviewing.

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# SENSORY EVALUATION AND CONSUMER TRUST: AN INNOVATIVE APPROACH FOR THE SUSTAINABLE SUCCESS OF AQUACULTURE IN THE EUROPEAN FISHEUTRUST PROJECT

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## Introduction

Consumer behaviour and preferences are key factors for the success of the aquaculture industry in meeting market demands and embracing innovative technologies. To understand and guide purchasing decisions, a synergistic approach has been developed that integrates virtual simulations and real-life experiences.

A central element of this strategy is sensory evaluation, which is crucial for deciphering consumption dynamics and defining effective market strategies. In the initial phase, highly qualified sensory experts conducted a Quantitative Descriptive Analysis (QDA) on various aquatic species (fish and shellfish), profiling their sensory characteristics in terms of taste, aroma, texture, and appearance. Subsequently, over 150 consumers for Italy, Denmark and Slovenia participated in culinary events and guided tasting sessions, expressing their preferences. This phase enabled the collection of valuable data on the most appreciated sensory attributes and identified significant differences among consumer groups with diverse habits and preferences.

Aquaculture faces the dual challenge of meeting the growing global demand and promoting sustainability. In this context, consumer trust is a cornerstone for fostering the adoption of innovations and ensuring the competitiveness of the sector. This study, as part of the FishEUTrust project, aims to strengthen trust in aquatic products through innovative interventions based on advanced sensory analyses and technological solutions.

## Materials and Method

The research was conducted in two distinct phases. Initially, a panel of sensory experts carried out a Quantitative Descriptive Analysis (QDA) on a selection of aquatic products, including fish and shellfish, to define their sensory profiles. The samples were evaluated according to standardised parameters related to taste, aroma, texture, and appearance. Subsequently, over 150 volunteer consumers from the three countries, participated in structured tasting sessions and culinary events, expressing their individual preferences through detailed questionnaires. The data collected were subjected to statistical analysis to identify correlations between sensory preferences and participants' dietary habits.

## Expected Results

The research results provide an in-depth understanding of the sensory characteristics most appreciated by consumers, such as taste, aroma, texture, and appearance, identifying the key attributes that drive their preferences and choices. The analysis highlights significant differences among various population segments influenced by factors such as dietary habits, cultural background, and personal experiences.

Through the analysis of sensory profiles of aquatic products, combined with direct feedback collected during guided tasting sessions, it has been possible to precisely define the characteristics that make products more attractive and desirable. This information is a crucial resource for developing market strategies aimed at effectively communicating sensory qualities and meeting consumer expectations.

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Furthermore, the integration of this knowledge with innovative technologies, such as virtual experiments and traceability systems, strengthens trust in sustainable aquatic products by promoting supply chain transparency and showcasing the value of responsible aquaculture practices. This synergistic approach seeks to encourage consumers to choose more sustainable options, driven not only by ethical principles but also by the high sensory quality of the products offered.

This research was conducted as part of the FishEUTrust project, which has received funding from the European Union's Horizon Europe programme under grant agreement no. 101060712.

# DEVELOPMENT OF A SENSORY PROFILE FOR DIPLOID MUSSELS FROM DIFFERENT EUROPEAN REGIONS: A COMPARATIVE STUDY USING QDA®

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## Introduction

Mussels represent a cornerstone of European aquaculture, appreciated not only for their nutritional value and sustainability but also for their distinctive sensory qualities, which are shaped by local farming practices and environmental conditions. This study aims to establish an objective and standardized sensory profile for diploid mussels from three major European production areas: Italy, Norway, and Ireland. The goal is to highlight region-specific organoleptic characteristics that can support product differentiation, enhance consumer perception, and strengthen market positioning.

## Materials and Methods

A Quantitative Descriptive Analysis (QDA®) approach was adopted, involving a trained panel of sensory assessors who evaluated key attributes (appearance, odour, flavour, and texture) based on a rigorously developed lexicon. Mussels were assessed in their fresh state shortly after arrival, and all panellists underwent specific training over several weeks to ensure consistent and reliable evaluations. Sensory assessments were performed under controlled laboratory conditions.

## Results

Preliminary findings reveal significant inter-regional differences in sensory profiles, particularly in terms of flavour complexity, colour intensity, and textural firmness. These differences appear to be strongly correlated with farming methods, feed availability, and environmental variables such as water temperature and salinity. By objectively characterizing the sensory identity of mussels from different European regions, this research offers valuable implications for quality control, product certification, and marketing strategies. Emphasizing the unique sensory attributes of mussels can play a pivotal role in increasing their appeal in high-value markets and fostering consumer trust in European aquaculture products.

# THE INFLUENCE OF 5-HTR AND NR3C1 POLYMORPHISMS ON THE AGGRESSIVE BEHAVIOR OF GILTHEAD SEABREAM (*Sparus aurata*)

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## Introduction

Gilthead seabream (*Sparus aurata*) is an important species for the Mediterranean aquaculture. Social structure significantly impacts animal welfare and productivity in intensive farming, underscoring the need to understand the genetic and neuroendocrine mechanisms behind these traits. Stress responses, aggression, and social hierarchy directly affect fish welfare and productivity, yet their genetic basis remains poorly understood. Studies in many animal models suggest that genetic variations (SNPs) in serotonin receptors (*5-htr1a, 5-htr2b*) and the glucocorticoid receptor (*nr3c1*) influence stress response. This study explored the genetic background of aggressive behavior in *Sparus aurata* (gilthead sea bream) by investigating SNPs in the *nr3c1*, *htr1a*, and *htr2b* genes using high-resolution melting (HRM) analysis. Seven primers were tested, all of which correlated with aggressive responses measured as total attack frequency, attacks before feeding and attacks post-feeding, as well as subordinate behavior in the tanks. Some SNPs were associated with aggression, underlining their potential role in stress response and social behavior regulation. These findings highlight the utility of HRM as a screening tool to identify molecular markers related with aggression.

## Materials and methods

Forty fish with an average body weight of 113.34 ± 18.19 gr and an average standard length of 15.92 ± 2.58 cm were kept in four tanks in a RAS system, and all water parameters were recorded daily. The average water parameters were: DO: 7.52 ± 0.30 mg/L, pH: 7.53 ± 0.45, salinity: 30.7 ± 2.3psu, water temperature: 23 ± 1.8 °C, and ORP: 226.5 ± 18.3mV. Fish were recorded with a video camera (1080p, 60fps) daily (5min) for seven days before and after feeding, and their behavior was assessed as follows: aggression, subordination, attacks before feeding, and attacks after feeding. Fin clips were used for DNA extraction according to standard protocols (Macherey-Nagel NucleoSpin Tissue kit). Sequences from chromosomes 5, 11, and 13 of the *Sparus aurata* genome (fSpaAur1.1 assembly), which contained 5HT2b, nr3c1, and 5HT1a gene sequences, respectively, were obtained from NCBI (NC_044191.1, NC_044197.1, NC_044199.1) and indexed with bwa index. For *in silico* SNP discovery, 22 fastq files ERR5296642-4, SRR9615483-4, SRR9615489-95, SRR9615501-3, SRR9615517-24 were acquired. Following quality filtering and annotation of the candidate polymorphisms, six HRM primers were designed. PCR reactions were carried out in a BIO-RAD CFX96 real-time cycler using the QIAGEN Type-it HRM PCR Kit. Genotypes were discriminated with a high-resolution melting analysis from 60 to 90°C with an increment of 0.2°C and confirmed with Sanger sequencing. GLM was used to examine the relationship between behavioral traits and genotypes.

## Results

After observing the aggressive behaviors between individuals, the attacks were analyzed, and most of the fish were categorized as aggressive and semi-aggressive, as in both tanks were observed fish with more aggressive tendencies than others were observed. There were on average 3,8 and 1,9 attacks before and after feeding, respectively. Around 26% of fish exhibited no aggression and remained completely inactive during the trials. A greater percentage of the population showed less aggression, with 37% (1-3 attacks), while 21% were considered very aggressive (more than 5 attacks), mostly in the prefeeding phase. The remaining 16% had responses that varied and did not easily fit into any category. HRM analysis revealed the different genotypes, which are presented in Figure 1.

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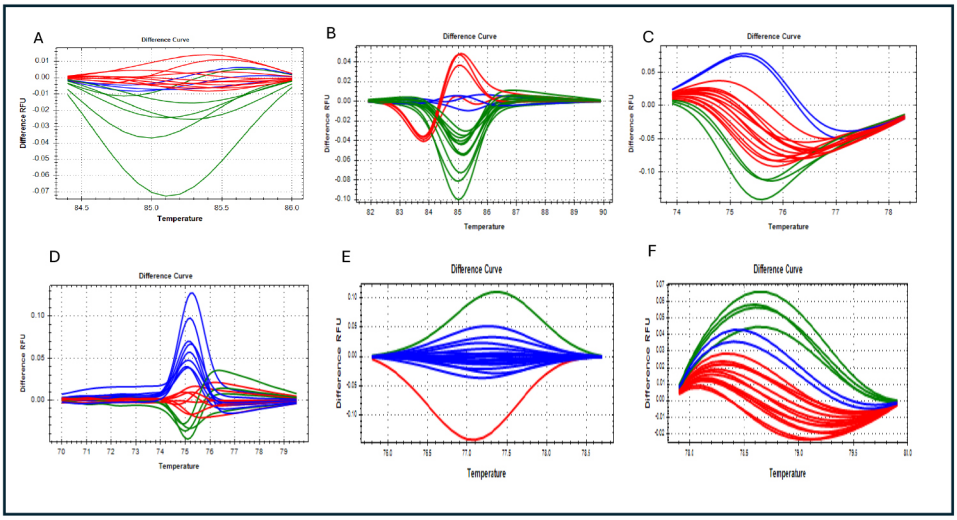


*Figure 1 HRM analysis depicting SNPs at genes nr3c1 (A,B), 5-htr1b (C,D) and 5htr2a (E,F)*

## Discussion

This study analyzed six SNPs in the *nr3c1*, *htr1a*, and *htr2b* genes in *Sparus aurata* using HRM and behavioral assays, revealing genotype-behavior correlations. Distinct aggression patterns matched stress-coping styles mediated by serotonin and cortisol pathways. *htr2b* polymorphisms showed linear aggression increases, while *htr1a* AG genotypes exhibited peak aggression, suggesting receptor sensitivity effects. In fish, aggression is correlated with serotonergic activity (Silva et al., 2012). Aggression was related to glucocorticoid receptor (*nr3c1*) variants, a gene that is regulated during physical stress (Malandrakis et al., 2016). HRM confirmed genotype-behavior links, with *htr2b* GG homozygotes exhibiting the highest aggression. htr2b polymorphism may have a functional role in regulating serotonergic signaling, which is known to affect vertebrate aggression (Delprato et al., 2018). These findings enabled the discovery of biomarkers for reduced aggression and improved welfare in aquaculture. The combined HRM-genotype-behavior approach advances precision in fish behavioral genomics.

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# LOW INTESTINAL INFLAMMATION MODEL (HP48) IN ATLANTIC SALMON (*Salmo salar*) AND INFLAMMATORY MITIGATION BY BACTOCELL

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## Introduction

Moderate levels of intestinal damage and inflammation are often seen in intensive fish aquaculture. This can be caused by stress, sub-optimal farming conditions or antinutritional factors present in plant meals. There is currently a lack of good models to explore these effects and how to mitigate the consequences. Thus, the aim of the present study was to develop a low-intestinal inflammation model using HP48, derived from solvent-extracted peeled soybeans and to test whether of the probiotic *Pediococcus acidilactici* CNCM I-4622 – MA 18/5 M (Bactocell) could diminish this effect.

## Materials and methods

Atlantic salmon (*Salmo salar*) post-smolts (averaging 106.8 ± 5.8 g) were assigned three feeding groups. The Control diet contained 18.08% soy protein concentrate (SPC) and for the HP48 diets, most of the SPC were exchanged with HP48 to induce low intestinal inflammation. The Bactocell diet was identical to the HP48 diet but was supplemented with 0.03% Bactocell (Lallemand Animal Nutrition, Blagnac, France). The fish were fed to excess over ~ two months and weight were recorded at the start, mid and end of the experiment. At the end sampling, mid- and hind-intestine were collected for analysis of histology, Ussing chamber and RNA sequencing.

## Results

Results revealed that fish fed the HP48 diet showed a reduced SGR (1.55 ± 0.04) compared to the Control diet (1.66 ± 0.03) ($p < 0.05$). The difference in SGR was greater in the first half of the experiment. The Bactocell diet showed intermediate growth (1.58 ± 0.03) which did not differ from the other groups. Histological assessment of the mid-intestine revealed few differences, except for wider lamina propria in the HP48 group, but transcriptomic and integrity data suggest that HP48 led to a disturbed mid-intestinal homeostasis with impaired cellular integrity and increased inflammation and cell turnover. Most of the transcriptomics effect were reversed with Bactocell. In the hind-intestine, HP48 led to several morphological changes, i.e., reduced vacuolisation and disrupted mucosal folds (Fig. 1) and upregulated immune related genes like CD99 and IL-17F. Bactocell alleviated several of the morphological changes, but did not reverse the gene expression.

## Discussion

Plant-based ingredients like soybean meal (SBM) are commonly used to study intestinal inflammation in Atlantic salmon (*S. salar*), but the resulting damage may be too severe for functional ingredients to mitigate (Krogdahl et al., 2003, Booman et al., 2018). In this study, a milder inflammatory model was developed using HP48 to better assess probiotic supplementation. Feeding HP48 led to lower SGR, especially early on, but recovery towards the end indicated mild and reversible damage (Ali et al., 2003). Bactocell supplementation improved SGR, suggesting a protective effect. HP48 caused impaired epithelial barrier function and low-level inflammation in the mid-intestine (Bakke-McKellep et al., 2007, Booman et al., 2018, Wu et al., 2018). Bactocell restored collagen and immune-related gene expression in this intestinal segment. In the hind-intestine, HP48 induced inflammation, including morphological changes and increased expression of in CD99 and IL-17F (Knudsen et al., 2007, Marjara et al., 2012). Bactocell supplementation mitigated the morphological effects but was not able to reverse the transcriptional effects. This could indicate that Bactocell increased colonisation of probiotic bacteria in the mid-intestinal segment compared to the hind-intestine. A dose-response trial would be interesting to investigate whether a higher inclusion of Bactocell would lead to more effects lower down in the intestine.

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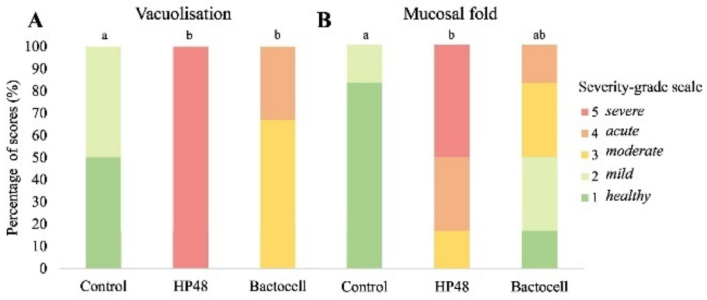


Fig. 1. Relative frequency distribution of A) visualization and B) mucosal fold scores in the hind-intestine per diet group (n=6 with 6 folds assessed per fish). Score 1 (green) represents large supranuclear vacuoles and thin, long folds, whereas score 5 (red) represents tissue disruption with fused mucosal folds.

## Conclusion

Overall, HP48 successfully induced mild intestinal inflammation, providing a better evaluation platform for functional feed ingredients. Probiotic supplementation with *Pediococcus acidilactici* (Bactocell) mitigated several of the negative effects, particularly in the mid-intestine.

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# COMPARATIVE ANALYSIS OF SWIMMING PERFORMANCE IN PIKEPERCH (*Sander lucioperca*) FROM RECIRCULATING AQUACULTURE SYSTEMS AND TRADITIONAL POND CULTURE

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**Introduction**

Pikeperch (*Sander lucioperca*) is a valuable aquaculture species with a steadily increasing market demand (Policar et al. 2016) frequency of fin erosion, skeletal and gill covers deformities, proximal composition of fish body with special emphasis on fatty acid contents in liver and muscle, oxidative stress and antioxidant response in gill, liver, muscle and intestine were analyzed and compared in exclusively RAS- and POND-cultured juveniles and juveniles produced with the combination of POND and RAS aquaculture in pikeperch (Sander lucioperca L.. Most pikeperch originating from intensive culture are intended for market-size production. Nevertheless, pond aquaculture is becoming increasingly interested in stocking material produced in recirculating aquaculture systems (RAS), mainly due to predictable production and logistical advantages. However, differences in rearing environments, such as RAS versus traditional pond culture, may significantly impact swimming capabilities and behavior in general, which are critical as a foundation for predatory efficiency and effective escape responses (Khendek et al. 2017). These traits are important for survival, influencing both pond aquaculture success and the effectiveness of restocking programs into natural habitats as in both scenarios fish is exposed to natural complex environment and conditions close to them (Juza et al. 2024). This study investigates how distinct rearing conditions influence the swimming performance of pikeperch, with particular emphasis on traits supporting predatory capacity and escape reflexes.

**Material and methods**

Pikeperch with an average body weight of 30 g, originating from two different rearing systems — recirculating aquaculture systems (RAS) and traditional pond culture — were used for the study. Prior to the swimming performance trials, fish were acclimated for two days to the experimental environment under controlled conditions. Swimming activity was assessed over a three-day period for each repetition. The trials were conducted in three identical flow arenas, allowing simultaneous testing of multiple individuals. Each experimental set consisted of three repetitions. Fish swimming behaviour was continuously recorded using infrared cameras, enabling observation during both day and night without disturbing the fish. The recorded footage was analysed using EthoVision XT software (Noldus Information Technology), providing detailed quantification of swimming parameters such as velocity, distance travelled, and activity patterns.

**Results and discussion**

Significant differences in swimming performance were observed between the two groups of pikeperch. Fish differed in average velocity, total distance travelled, and overall activity levels throughout the recording periods. These differences were consistent across all repetitions and were evident during both daytime and nighttime observations.

The variation in swimming performance likely reflects the impact of rearing conditions. Environmental factors such as water movement and habitat complexity during the early stages of development can influence muscular development, stamina, and behavioral patterns. Fish reared under more dynamic conditions may develop enhanced swimming abilities, which can be advantageous for survival after release. These results highlight the importance of considering environmental conditioning in aquaculture practices, particularly for species intended for stocking into natural or semi-natural environments.

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# USE OF DIFFERENT APPROACHES FOR THE DESIGN OF A SUBUNIT VACCINE AGAINST AEROMONAS SALMONICIDA IN TURBOT (*Scophthalmus maximus*) AND SOLE (*Solea senegalensis*)

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**Introduction**

This project aims to formulate a broad-spectrum subunit vaccine against A. salmonicida, focusing on two high-value species: turbot (Scophthalmus maximus) and sole (Solea senegalensis). To this end, candidate immunogenic proteins were identified using two complementary approaches: (1) a proteomic analysis based on the shaving technique applied to seven strains isolated from both host species, and (2) a bioinformatic screening based on published proteins of A. salmonicida subsp. salmonicida. These results were complemented by a review of recent literature on proteins with immunogenic potential. The selected proteins were expressed in two different experimental formulations, which were evaluated through immunisation trials.

**Materials and methods**

Seven different strains of A. salmonicida were used, each isolated from different individuals of turbot and sole (see Table 1). The shaving technique was applied to each bacterial sample, adding trypsin at 5 $\mu$g/mL (Olaya-Abril et al., 2014). Finally, the samples were completely dried in a SpeedVac concentrator. The peptides generated were analysed by ultra-high-pressure liquid chromatography (nanoElute, Bruker) coupled to a high-resolution timsTOF PRO mass spectrometer (Bruker). The proteins were detected using the UP000000225 database. The detected proteins were analysed using bioinformatic tools, with the aim of predicting their subcellular location, presence of signal peptide, and immunogenic potential (Razali et al., 2023). This screening allowed us to prioritise candidate proteins with the highest probability of exposure and recognition by the host immune system.

Finally, ExeD was selected as the main candidate. Its sequence was cloned into a pet20b plasmid and introduced into the E. coli BL21(DE3) strain. The recombinant protein was expressed from a culture in LB medium (50 mL) and isolated using an inclusion body extraction protocol (Novagen, pET System Manual, 11th edition). In addition, the VapA protein was considered due to its relevance described in the literature, despite having been excluded from the pool of candidate proteins after searching using both approaches. For its purification, strains B, D, E, F, and G of A. salmonicida were used in a glycin solubilization based method (Bjørnsdottir et al., 1992). Three infection challenges were performed 1 and 3 months after vaccination with vapA, as detailed in Table 2, and administered intraperitoneally, while exeD is pending validation.

**Results**

ExeD was the protein chosen after shaving and bioinformatic screening. Although no challenge has been performed for exeD, it should be noted that this is an interesting protein involved in the type II secretion system, which is important in the secretion of virulence substances in Gram-negative bacteria. This protein forms an important multimeric channel in this task.

In the challenge 1 month after vaccination, the first group showed an RPS of 30% and a mortality rate of 48%, compared to an 80% mortality rate in the control group. The second and third groups showed an RPS of 50% and 33.33%, and a mortality rate of 12% and 16%, compared to the 24% mortality rate of their control group. In the challenge 3 months after vaccination, the first group showed an RPS of 50% and a mortality rate of 16%, compared to the 80% mortality rate of its control group.

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**Table I: Strains of *A. salmonicida* used to search for immunogenic proteins using the shaving technique**

| ID cepa | Subspecies | Host |
|---|---|---|
| A | *A. salmonicida subsp. achromogenes* | Turbot |
| B | *A. salmonicida subsp. achromogenes* | Turbot |
| C | *A. salmonicida subsp. salmonicida* | Turbot |
| D | *A. salmonicida subsp. achromogenes* | Sole |
| E | *A. salmonicida subsp. achromogenes* | Sole |
| F | *A. salmonicida subsp. achromogenes* | Turbot |
| G | *A. salmonicida subsp. achromogenes* | Turbot |

**Table II: Groups used in vaccination challenges**

| | Host | *A. salmonicida* strains | Number of fishes | Weight per fish(g) | ug of protein per fish |
|---|---|---|---|---|---|
| **1st group** | Sole | D, E | 150 | 15 | 24 |
| **2nd group** | Turbot | F | 175 | 60 | 25 |
| **3rth group** | Turbot | B, G | 175 | 60 | 50 (25 each strain) |
| **Control 1st group** | Sole | - | 250 | 15 | - |
| **Control 2nd and 3rth group** | Turbot | - | 250 | 60 | - |

## Conclusion

The results obtained in trials with the VapA protein indicate a significant protective immune response in sole and turbot, with notable reductions in mortality (up to 64% in some groups) and RPS values reaching 50% even three months after vaccination. These data suggest that VapA is a promising candidate for the development of subunit vaccines against A. salmonicida in species of high commercial value. Although the trial with the ExeD protein is pending, its role in the type II secretion system and its exposure on the bacterial surface make it a target of high interest for future evaluations. The combination of proteomic and bioinformatic approaches has proven effective for the identification of candidate antigens, and their validation in field trials could contribute to improving health and productivity in aquaculture, reducing dependence on antibiotic treatments.

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# POTENTIAL OF THE GREEN SEAWEED *Codium* FOR HUMAN FOOD PRODUCTION: FROM LINE SEEDING TO BIOMASS PROCESSING

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## Introduction

Seaweeds have a longstanding tradition in their use for human consumption in many Asian countries. In the western world, their use has for long been limited to limited coastal areas and has been associated for a long time with episodes of hunger and war. This view has been changing drastically as the need for new prime matter for human food production is has been growing. Seaweeds are now also in the western world increasingly being considered as a more sustainable, high quality food source.

Against this background, several R&D projects have been executed in the last 5 years, focusing on cultivation of seaweeds and their use in human food production, such as the Spanish INNOVALGA project (2020-2023) and the EU Horizon Europe project NOVAFODIES (2023-2026) that both aimed to stimulate diversification, efficiency, innovation, and upscaling of European micro- and macroalgae solution.

In this contribution we will present an integral overview of a potential production chain based on the green seaweed *Codium*, from line-seeding to biomass composition and production of a food prototype. The genus *Codium* has a worldwide distribution and a traditional use in various countries both within and outside Europe and various interesting functional properties. Its taste is generally considered subtle, reminiscent of crustaceans. It is therefore considered to be promising ingredient for the food of the (near) future.

## Material and Methods

Locally collected specimens of the species *Codium taylorii* were maintained in a climate room (20 °C) in plastic containers in fresh seawater (weekly renovation) at minimum light (< 20 µmol photons $m^{-2}$ $s^{-1}$) and under constant aeration. Biomass samples were analysed for their nutritional composition, antioxidant properties and antimicrobial properties. To optimize low-cost cultivation of *Codium*, seeding trials were carried out, studying the effects of temperature, irradiance, and substrate type for biomass seeding on lines. A fully factorial experiment has been carried out considering two light intensities (60 and 100 µmol photons $m^{-2}$ $s^{-1}$) and two temperatures (15 and 20 °C) and three substrate types (cotton, hemp and polypropylene ropes). Seeded lines were deployed in earthen ponds to study outgrowth. The seaweed was subjected to shelf-life trials in which fresh biomass stored directly or in vacuum packaging in the refrigerator. Organoleptic properties and food microbial analyses were carried out at different time intervals to determine shelf-life. Finally, a food prototype product was developed in the form of fish-filled ravioli-type pasta. Freshly harvested algae were added to the pasta-dough in differ recipe formulations and products were subjected to organoleptic and biosecurity tests.

## Results and discussion

Freshly harvested *Codium* biomass can be maintained for more than 6 months with minimal maintenance effort as described. With respect to composition, compared to other macroalgae, *C. taylorii* had a relatively high water content (92% DW), whereas protein content was relatively low (8-10% DW). However, literature values for the species list values up to 18% and therefore claim to be high fibre and high protein biomass according EU regulations (EC Regulation No. 1924/2006). The percentage of essential amino acids for all samples was approximately 40% and generally favourable for human nutritional needs.

Mean antioxidant capacity of *Codium* was comparable to reported values of other green seaweeds and consistent across TEAC and ORAC test methods. No antimicrobial properties of the algae were detected were detected using the agar well diffusion method, however, watery extracts from *Codium* presented significant antimicrobial activity against the food pathogen *Staphylococcus aureus*.

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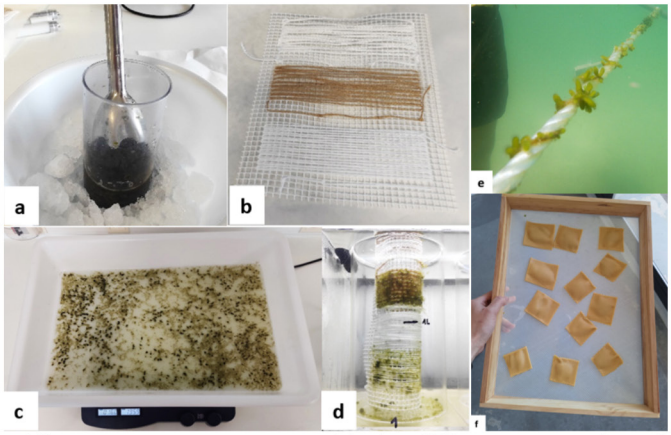


Figure 1: Shredding process (a), support with the different types of seeding yarns (b), shredded mixture sieved and ready to incubate the support with the yarns (c), aquarium with the yarns arranged cylindrically (d) seeded line deployed for outgrow (e), and ravioli-type pasta prototype product (f).

With respect to the cultivation trials, successful seeding of the lines was obtained. Both temperature and substrate significantly affected appearance of *Codium* Forming Units (CFU) on lines, whereas irradiance was significant in second-order interactions. The highest number of CFUs (11 units/cm) occurred in the high-temperature, low-irradiance treatment for hemp yarn. It was concluded that substrate was the main factor determining fixation success, moderated by temperature and light interactions. Outgrowth in earthen ponds proved successful initially, although modifications had to me made to the seeding lines.

Shelf-life tests indicated limited storage time (1 week) of fresh *Codium* under refrigerated conditions, although vacuum packaging seemed to slightly prolong shelf life. Although counts of major food pathogens were well below legal limits, general count of aerobic bacteria was too high and organoleptic properties decreased drastically after 1 week. First pasta dough prototype products elaborated with *Codium* produced satisfactory results with respect to product structural and organoleptic qualities. The final product was slightly orange coloured, probably due to relatively high β-carotene contents of the *Codium*.

It is concluded that *Codium* shows considerable promise for cultivation as prime matter for human food ingredients with prospects of efficient cultivation. Future studies should concentrate on the outgrow phase. Regarding biomass processing, further studies on the effects of storage methods on nutritional qualities and alternative storage methods to ensure longer shelf life are required.

**Acknowledgments/Funding**
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# EFFECTS OF SUBSTRATE TYPE AND DIETARY ENRICHMENT ON THE GROWTH PERFORMANCE OF THE SEA CUCUMBER *Holothuria arguinensis* (Koehler & Vaney, 1906) IN RECIRCULATING AQUACULTURE SYSTEMS

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Sea cucumbers are experiencing increasing demand, particularly in Asian markets, which has led to their high economic value (Purcell,*et al.,* 2023). As a result, many species are subject to overexploitation, often through illegal, unreported, and unregulated (IUU) fishing practices and/or poor fishery management. Aquaculture represents a sustainable alternative to prevent further depletion of wild sea cucumber populations (Sousa *et al*., 2023; Domínguez-Godino and González-Wangüemert, 2019; Félix *et al.,* 2018).

Recent advances in culture techniques for the genus *Holothuria* have improved methods for reproduction and captive rearing. Key developments include spawning induction (Venancio et al., 2021), larval and juvenile management (Madruga et al., 2023; Sousa et al., 2023) and specialized feeding strategies (Domínguez-Godino and González-Wangüemert, 2019a; Rodrigues et al., 2023), which have led to optimized survival and growth rates. Factors such as substrate conditions, diet supply and stocking density are determinants of culture success (Domínguez-Godino et al., 2015). To date, no commercial feed has been patented for sea cucumbers, and the development of effective diets remains an active area of research.The study aimed to evaluate the impact of different substrate and diet combinations on the growth performance of *H. arguinensis* cultured in a recirculating aquaculture system (RAS).

Individuals of *H.arguinensis* (n=24) were collected from the wild and acclimated in indoors tanks for 48h without diet enrichment. The experiment was divided into two phases: i) assessment of three types of substrates (sand, mud and a mixture of both) on growth and survival; ii) evaluation of three feeding regimes were evaluated: T1 (no enrichment), T2 (50:50 mixture of *Ulva* sp. and commercial feed), and T3 (replacement of *Ulva sp*. with *Chaetomorpha sp*.). Experimental tests were carried out with the main objective of determining the optimal substrate and diet combinations to maximize growth performance in a RAS system.

In both cases, the Specific growth rate (SGR) (Ricker, 1979) and the mortality rate (%) were analyzed and evaluated to determine the culture feasibility. Organic matter content in substrate was determined using the loss on ignition (LOI) method (%). Statistical analysis was performed using R-Studio (R version 4.2.1). Differences among treatments were evaluated using one-way ANOVA followed by post-hoc tests, with significance set at $P < 0.05$. Substrate type did not significantly affect growth, however the sand treatment showed greater variability in SGR. The lowest growth was recorded in the control (no substrate), with a mean SGR of -0.10 ± 0.11 %/day, indicating limited development in the absence of substrate (Fig. 1). The Overall mortality rate was low (one individual only). LOI analysis revealed higher organic matter content in mud sediments (1.2971% and 1.1522%) compared to sand (0.1554%). This, along with the average SGR for mud (0.29 ± 0.35 %/day), led to the selection of mud as the substrate for the second experimental phase, testing diet supply only.

Among the three dietary regimes tested, the treatment containing *Chaetomorpha* sp. and commercial feed (T3) yielded the highest growth rate (0.56 ± 0.65 %/day), with statistically significant differences compared to the control group (Fig. 2). No mortality was recorded during this phase.

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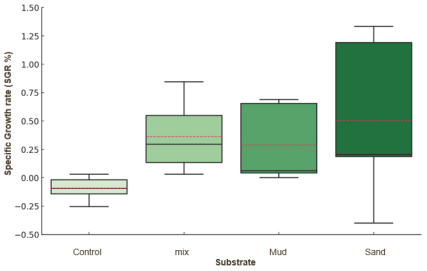


**Fig 1.** Specific Growth rate (%/d) of *H. arguinensis* under four culture conditions varying types of substrates (sand, mud, and 50:50 sand & mud)

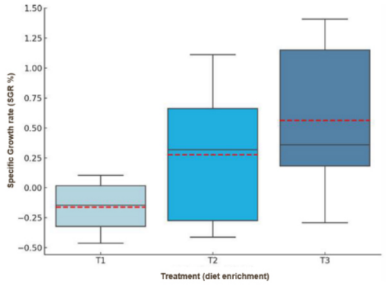


**Fig 2.** Specific Growth rate (%/d) of *H. arguinensis* under three feeding regimes: T1 (no enrichment), T2 (50:50 mixture of *Ulva sp.* and commercial feed), and T3 (replacement of Ulva sp. with *Chaetomorpha sp.*

The results emphasized the importance of the presence of sediment in the cultivation systems of *H. arguinensis*. Both muddy and sandy sediments and their mixture proved to be suitable substrates for animal growth, without rendering significant differences between them. Both have their advantages; mud provided a higher organic matter content, offering a potential nutritional benefit, while sand contributed favorable physical conditions for development. Dietary enrichment demonstrated to be a key factor in enhancing growth of *H. arguinensis*. The combination of *Chaetomorpha sp*. and commercial feed (T3), particularly when used with a muddy substrate, significantly improved growth compared to the control.The requirement for substrates to support optimal growth presents challenges for indoor tank-based systems. Consequently, alternative culture environments such as earthen ponds should be explored to facilitate scaling and improve operational efficiency in the cultivation of this species.

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# DYNAMICS OF LIPID AND FATTY ACIDS IN CARP GILL-FILTERABLE PLANKTON CONSORTIA OF TEMPERATE EUROPEAN FISHPONDS: SIGNATURE TRENDS THAT CAN BE USED FOR PLANNING LC-PUFA MINING

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## Introduction

Landlocked regions such as Czechia exhibit critically low omega-3 indices, correlating with high cardiovascular mortality and ageing related disorders. There is an import dependency on long-chain omega-3 polyunsaturated fatty acids (LC-PUFAs), essential for cardiovascular and cognitive health, from overexploited marine resources. While microalgae and genetically modified vegetable oils present potential solutions, their scalability and regulatory limitations hinder widespread adoption. In contrast, Central European semi-intensive fishponds—regulated under EU water directives—harbor abundant plankton-derived LC-PUFAs (800-1600 mg EPA+DHA 100 g dry matter$^{-1}$), yet their trophic transfer to farmed fish remains inefficient. Common carp (*Cyprinus carpio*), predominantly fed cereal-based diets deficient in PUFAs, exhibit poor retention of LC-PUFAs in fillets, despite plankton availability. Also, application of any farming solution to improve LC-PUFA transfers from pond food web to pond fish is discouraged by nuanced arguments such as '*each year is different*'' or '*each pond is different*'. In this research, we explored whether such arguments are true or unfounded.

This study investigates the interannual as well as seasonal dynamics of dry matter, lipids and fatty acids in common carp gill filterable (>200 $\mu$m) plankton consortia in relation to physicochemical parameters, fishgrazing pressure, and human intervention such as supplementary feeding. By assessing these dynamics, we evaluate plankton nutritional quality to develop pond feeding strategies, improve LC-PUFA retention and sustainable land-locked mining of LC-PUFA from the pond farmed fish. In a way, to develop nature-based solutions one first have to understand the nature itself.

## Material and method

A study was conducted in nine uniform (0.2 ha, 1 m deep) experimental ponds in Vodňany, Czechia (49°09'24.9"N, 14°09'54.2"E; 404 m a.s.l.), stocked with Cyprinus carpio (471.3 kg ha$^{-1}$) and differentially fed (control ponds: 563 kg wheat pond$^{-1}$ throughout season, n=3 replicates; treatment ponds: 123 kg wheat pond$^{-1}$ during April-June + 432 kg plant-based pellet pond$^{-1}$ during July-October, n=6 replicates, half hand-fed and other half by demand feeder). The plant pellets were non-refined and agri-food by-products based, with protein content ~28% and lipid ~3.5%. From April to October 2024, monthly sampling included water quality, climatic variables, submerged temperature loggers, fish biometrics, and net plankton consortia (>200 $\mu$m) was collected that pond carp generally acquires by gill filtration while predominantly grazing on the pond pelagic food web (that is the larger feeding area/ surface area, compared to feeding surface area of pond bottom or margin). Lyophilized (14 hours main drying + 10 hours final drying) plankton consortia (May–October) were analyzed for dry matter (DM), lipid composition, and fatty acids. Comparative analysis with 2022 data was performed, followed by seasonal trend visualization and Pearson's two-tailed correlation (OpenAI Data Analyst 4o, R).

## Results

Between 2022 and 2024, the annual mean lipid concentration (% DM) of gill-filterable plankton consortia differed significantly ($p<0.05$), but the monthly trend or seasonal progression remained consistent (Month × Year: p = 0.998) between years. Despite different feed formulations applied to pond groups, plankton lipid and dry matter content showed no statistically significant difference. Both DM (mg l$^{-1}$) and lipid ($\mu$g l$^{-1}$) concentrations peaked in June–July, reaching minima in August–October, a pattern congruent with Cladocera abundance (a good marker) and exhibiting moderate positive correlation ($r$= 0.30-0.42, $p<0.01$). Chlorophyll-a (a eutrophication proxy) and temperature displayed a moderate inverse correlation with

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lipid quality and availability. An inverse trend was observed between storage lipids (e.g., triacyclglycerols) and structural lipids (e.g., phospholipids) over the season. As the season progressed, the pelagic food web seemed to deteriorate in storage lipid classes and preserve the critical structural lipids; and a strict homeostasis on cholesterol throughout season. LC-PUFAs like eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6) exhibited similar trends in the pond pelagic food base (gill-filterable). They were abundant (mean±standard error) during the beginning of season (May-July;2.62 ± 0.36 $\mu$g EPA $l^{-1}$, 24.35 ± 4.21 $\mu$g DHA $l^{-1}$, 17.41 ± 2.29 $\mu$g ARA $l^{-1}$), but declined during end of season (August-October; 1.3 ± 0.24 EPA $\mu$g $l^{-1}$, 12.35 ± 2.37 $\mu$g DHA $l^{-1}$, 4.91 ± 0.98 DHA $\mu$g $l^{-1}$ ). Additionally, the alpha-linolenic acid (ALA, 18:3n-3) requirement for pond carp is not fulfilled during August-September (modelled on a system or pond-wide scale). Which means during these months carp may not have the luxury to endogenously fortify LC-PUFA from an excess of ALA pre-cursor. The (SFA+MUFA): PUFA ratio (PUFA sparing proxy) was elevated in beginning of season (May–July) and declined thereafter, implying PUFA or omega-3 sparing effect may be compromised too in second half of the season (if no non-protein energy is fed to ponds). Concurrently, the n-3: n-6 PUFA ratio decreased progressively from May, reaching minimum in July–August before rebounding in September–October. Overall results suggest that the period of late July to September is a critical period, when both the supply and endogenous fortification of LC-PUFA is quite constrained in temperate European fishponds.

**Discussion**

Based on 2022 and 2024 observations, we rebut the nuanced arguments mentioned in the introduction. Results suggest both lipid quantity and quality in the carp gill-filterable pond pelagic food web predictably deteriorate mid-season– typically during late July to early September. It happens irrespective of the year or ponds with different supplementary feed choice. The lipid and fatty acids dynamics in temperate European semi-intensive fishponds exhibit a signature trend that can be used to plan feeding solutions in the temperate Central Eastern European region. Cladocerans, a reliable marker of lipid and LC-PUFA availability in ponds, decline seasonally (Aug–Oct) due to intensified fish predation and eutrophication-induced shifts in food resources, whereas copepod abundance remains stable (although it does not cover the missing lipids in food web due to missing cladocerans). Coupled with diminished lipid availability and LC-PUFA constraints during pre-harvest (Aug-Oct), this obviously results in low LC-PUFA concentration in carp fillets harvested during November (100-150 mg EPA+DHA 100 $g^{-1}$). These findings underscore the necessity to prolong Cladocera abundance by promoting submergent vegetation as forage for zooplankton, and end-of-season supplementary feeding with energy-dense (lipid energy) formulations to mitigate LC-PUFA catabolism. Late July to September is identified as a critical window for balanced nutritional interventions in ponds in terms of lipids or finishing feeding strategies.

**Acknowledgement**

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# AQUAVAX PROJECT "STANDARDISATION, DISTRIBUTION AND EVALUATION OF THE EFFICACY OF AUTOGENOUS VACCINES IN THE FISH SECTOR AS A SUPPLY CHAIN STRATEGY FOR THE CONTAINMENT OF BACTERIAL DISEASES AND THE REDUCTION OF ANTIMICROBIAL USE"

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Aquaculture has rapidly become one of the fastest-growing food industries and represents a vital source of protein for populations worldwide. The expansion of finfish aquaculture is closely associated with the development of intensive farming systems. In these facilities, fish are reared in different tanks/net cages, which can under certain conditions facilitate the rapid transmission of infectious agents and can lead to significant economic losses. Antimicrobials play a crucial role in the treatment of symptomatic bacterial fish diseases. However, their incorrect use in the past has contributed to the emergence of antimicrobial-resistant pathogens, posing potential risks to both human health and environmental sustainability. Vaccination strategies have demonstrated a positive impact in reducing the risk of antibiotic-resistant bacterial strains. However, compared to other livestock sectors, the availability of commercial vaccines for aquaculture remains limited. Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), a public institute belonging to the Italian Ministry of Health, has established a certified vaccine production laboratory dedicated to autogenous vaccines, and leads the AquaVax project funded by the Italian Ministry of Agriculture and Forestry (MASAF).

AquaVax is structured into four operational phases, involving specific Operating Units (O.U.s).

**Phase 1: Enhancement of Production Performance and Standardization of Antigen and Autogenous Vaccine Production Processes for Fish**
This phase focuses on implementing and standardizing production protocols mainly for *Yersinia ruckeri* biotype 2 and *Lactococcus garvieae* formulations, laying the foundation for developing monovalent vaccines (*e.g.,* an autogenous vaccine for lactococcosis in marine species) as well as a bivalent formulation, which is in high demand among trout farmers. To improve both the quality and quantity of vaccine provision, a series of trials will be conducted to ensure standardized protocols. Furthermore, new chemical agents for pathogen inactivation and plant-based oil adjuvants will be tested. The "water-in-oil" emulsion process will also be standardized. All formulations will undergo both *in vitro* and *in vivo* testing to ensure they are completely non-toxic to fish.

**Phase 2: Production of Autogenous Vaccines Targeting Major Bacterial Diseases**
Building on the outcomes of Phase 1, priority will be given to developing innovative vaccine prototypes based on the needs of fish farmers and on the prevalence of bacterial diseases for which no commercial vaccines are currently available.

**Phase 3: Training of Technical Staff for the Standardization of Vaccination Protocols and Administration Procedures**
Theoretical sessions will be complemented by on-site, hands-on and / "on-the-job" training at selected farms, involving all relevant staff. These activities will be carried out in collaboration with industry experts and/or fish farmers association to identify suitable training sites and coordinate the implementation process. This phase is essential to the success of the project, as field vaccination practices are influenced by several variables, including farm management, disease seasonality, staff availability and staff experience.

**Phase 4: Evaluation of the Immune Response in Vaccinated Fish Populations According to the Developed Protocols**
The Department of Agri-Food, Environmental, and Animal Sciences (University of Udine) with a long expertise on farmed fish immunology will assess the effectiveness of the vaccination protocols developed in the above metioned phases. These evaluations will take place at selected farms, in close collaboration with farm veterinarians and fish farmers.

**Conclusion**
The development of fish vaccines requires strong collaboration between research institutions, pharmaceutical industry, fish farmers and regulatory authorities. An effective vaccination strategy has proven to significantly reduce the impact of major bacterial diseases and to lower the risk of antibiotic-resistant in aquaculture.

# PRELIMINARY STUDIES ON THE EFFECTIVENESS OF ESSENTIAL OILS AGAINST *Lactoccoccus garvieae* ISOLATED FROM MARINE FISH SPECIES IN ITALY

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**Introduction**

Since their discovery, antibiotics have been used to treat infections caused by bacteria, in human medicine to heal people, but also to save both pets and farm animals in the veterinary field. Unfortunately, in recent years, their excessive and often inappropriate use has led to the development of antibiotic resistance, meaning that certain antibacterial molecules are no longer effective because the bacteria have been able to develop and/or acquire genes that can render the mechanism of action with which those specific molecules were able to act on the target ineffective.

Aquaculture farmed fish are no exception. In the event of disease outbreaks, they can be treated with antimicrobials, and this sector is also experiencing antibiotic resistance and treatment ineffectiveness, resulting in animal losses and significant economic damage to the production sector.

Currently, in addition to vaccination, probiotic microorganisms and new, more natural molecules are being sought to help fightening bacterial infections while reducing the use of antibiotics. Among these substances, essential oils seem to have emerged in recent years as a valuable tool in the fight against the spread of bacterial diseases.

**Materials and methods**

Various essential oils (oregano, lavender, thyme, melaleuca alternifolia, cajeput, lemongrass, cinnamon) or mixtures thereof were tested to assess whether these substances could have a bacteriostatic and/or bactericidal effect against *L. garvieae* isolated from marine fish species farmed in Italy.

The study was conducted following the guidelines of the Clinical & Laboratory Standards Institute (CLSI) by bringing a suspension of the microorganism under examination into contact with different concentrations of essential oil to find the MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration). The MICs of the different strains under examination were also established with the antibiotic molecules currently used in aquaculture.

**Results**

The initial results show that *L. garvieae* is resistant to individual essential oils. In fact, at high concentrations of oil, the strains under examination were found to be viable and it was not possible to determine the MIC and MBC. Cinnamon and a mixture of oils, on the other hand, proved to be more effective in treating *L. garvieae*, as it was possible to establish the MIC and, in some cases, the MBC.

These are preliminary results. In the future, other essential oils and mixtures will be tested on *L. garvieae*, an emerging pathogen in Italian marine aquaculture, and the sensitivity to essential oils will be analysed on other pathogenic strains isolated from marine species such as *V. anguillarum*, *V. harveyi*, *T. maritimum* and *P. damselae*.

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# PROTECTIVE EFFICACY OF CAMP VAC® STREPTOMUNE VACCINE AGAINST INFECTIONS CAUSED BY STREPTOCOCCUS AGALACTIAE SEROTYPES IB AND III IN NILE TILAPIA (*Oreochromis niloticus*)

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**Introdução**

Streptococcosis, caused by *Streptococcus agalactiae* (serotypes Ib and III), poses a serious threat to tilapia farming, causing significant economic losses (Delphino et al., 2019). Currently, the main therapeutic measure used during bacterial outbreaks is antibiotics, which can lead to bacterial resistance and even antimicrobial residues in fish when used indiscriminately (Milijasevic et al., 2024). An alternative to antibiotics for preventing infections is fish vaccination, which induces specific antibodies, conferring protection and safety to the immunized host, as well as economic benefits (Rivas et al., 2023). This study evaluated the efficacy of the CampVac® Streptomune vaccine in preventing experimental infection, conferred immunity, and production performance of Nile tilapia (*Oreochromis niloticus*) using highly virulent Brazilian strains.

**Materials and Methods**

Six hundred seronegative juveniles (30 ± 0.1 g) for *S. agalactiae* were used, distributed into 15 tanks (40 fish/tank). Experimental groups included: GCN1 (unchallenged negative control), GCP2 (serotype Ib-challenged positive control), GCP3 (serotype III-challenged positive control), GV4 (vaccinated/challenged with serotype Ib), and GV5 (vaccinated/challenged with serotype III). Vaccination (Day 0) was administered intraperitoneally (0.05 mL/fish). Challenge (Day 30) used established LD50: $4.6\times10^7$ CFU/fish (serotype Ib) and $1.6\times10^7$ CFU/fish (serotype III), administered via intracelomic (50%) and intramuscular (50%) routes. Water quality parameters (pH: 7.8±0.2; $O_2$: 7.1±1.5 mg/L; ammonia: 0.1±0.01 mg/L; temperature: 25.1±0.6°C) were monitored daily, maintaining optimal conditions. Mortality and clinical signs were assessed for 30 days post-challenge. Liver, spleen, and brain samples were collected for bacterial count and molecular confirmation by PCR and sequencing. Relative Percent Survival (RPS) was calculated as RPS = [1 - (% mortality treatment / % mortality control)] × 100. Data were analyzed using SAS (ANOVA, Fisher's exact test; Student's T-test; $p<0.05$).

**Results**

The vaccine demonstrated high protective efficacy: Survival in vaccinated groups (GV4: 97.5±2.5%; GV5: 94.2±6.3%) was significantly higher ($p<0.001$) than in positive controls (GCP2: 61.7±1.4%; GCP3: 53.3±8.0%) (Table 1). RPS was 93.6±3.6% (serotype Ib) and 88.5±7.3% (serotype III). A significant reduction ($p<0.001$) in bacterial load was observed in tissues of vaccinated fish, notably in the liver of GV4 ($2.16\times10^2$ CFU) compared to GCP2 ($5.51\times10^5$ CFU). GV5 showed absence of bacteria in the brain and spleen. Regarding production performance (Day 60), vaccinated fish exhibited higher weight gain (GV4: 67.9±8.5 g; GV5: 54.5±1.8 g) versus non-vaccinated challenged groups (GCP2: 38.1±4.4 g; GCP3: 31.7±3.1 g) and better feed conversion ratio (GV4: 1.70±0.14; GV5: 1.93±0.26 vs. GCP2: 3.33±0.48; GCP3: 3.85±0.22).

**Discussion**

Robust protection (>88% RPS) against both serotypes was accompanied by a drastic reduction in bacterial load in key organs, with the notable absence of bacteria in the brain of vaccinated fish suggesting blockage of neural dissemination. This RPS value is higher than the 59.14% reported by Rivas et al. (2023). The superior production performance of vaccinated groups (72-78% higher weight gain than infected controls) indicates that immunization minimizes energy diversion towards inflammatory responses, optimizing growth (Delphino et al., 2019). Despite the higher virulence of serotype III ($LD_{50}$ threefold lower than Ib), the vaccine-maintained survival >94%, demonstrating efficacy against Brazilian strains.

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**Table 1. Survival (SUR), Relative Percent Survival (RPS), and Performance of Nile tilapia (30 days post-infection) inoculated with *S. agalactiae* Serotype Ib and Serotype III.**

| Group | SUR (%) | RPS (%) | Weight Gain (g) | Feed Conversion Ratio (g/g) |
|---|---|---|---|---|
| GCN1 | 98.3±2.9a | - | 99.15±2.70a | 1.38±0.03 |
| GCP2 | 61.7±1.4b | - | 38.11±4.37d | 3.33±0.48 |
| GCP3 | 53.3±8.0b | - | 31.65±3.13d | 3.85±0.22 |
| GV4 | 97.5±2.5a | 93.6±3.6 | 67.89±8.47b | 1.70±0.14 |
| GV5 | 94.2±6.3a | 88.5±7.3 | 54.45±1.75c | 1.93±0.26 |
| *P-value* | *0.001** | *0.593#* | *0.001** | *0.001** |

Mean ± standard deviation. Values within a column followed by different superscript letters differ significantly (P<0.05) by *Fisher's Exact Test and #Student's T-Test.
Source: Authors

## Conclusions

CampVac® Streptomune is highly effective against *S. agalactiae* serotypes Ib and III in Nile tilapia, demonstrating: (1) >35% reduction in mortality compared to infected controls; (2) Significant tissue protection (bacterial load ≤$10^2$ CFU in vital organs); (3) Maintenance of production performance (FCR <1.93 vs. >3.33 in infected groups); (4) Proven efficacy against highly virulent native strains. This vaccine represents a strategic tool for the sustainable management of streptococcosis in tilapia farming, offering both economic and health benefits.

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# MODELING PRODUCTIVITY AND PROFITABILITY FOR SMALLHOLDER TILAPIA AND SHRIMP POLYCULTURE IN JAVA

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## Introduction

Aquatic polyculture is re-emerging globally as a promising system to optimize yield and profit (Knowler et al., 2020), including in Java, Indonesia. Among smallholder farmers in Java, tilapia-shrimp polyculture is one of the chosen species combinations (Setyawan et al., 2022a). However, optimizing profit in tilapia-shrimp polyculture systems remains a challenge due to the underexplored effects of management decisions on species interactions, which influence productivity and profitability. The objectives of this study are to (1) develop a multispecies bioeconomic model for pond production of tilapia and shrimp that incorporates species interactions and (2) validate the model outputs.

## Materials and methods

The multispecies bioeconomic model was developed through a literature review and insights from a scoping visit in Java. Model inputs are based on management parameters that farmers can influence: feed, stocking density, stocking weight, and culture duration. The model operates on two levels: (1) the individual species level which produces daily weight gain as output, and (2) the pond level which incorporates interaction effects from the other species and produces biomass, daily mortality, and cumulative mortality for each species as outputs. The final outputs are total production and operating profit expressed in USD/$m^2$/year. The model was programmed in R software version 4.3.2 (R Core Team, 2023) as a simulation tool. Expert validation of model projection was conducted for six scenarios (Table 1). Four scenarios with average feeding rate and (1) low tilapia and low shrimp density (LT-LS), (2) low tilapia and high shrimp density (LT-HS), (3) high tilapia and low shrimp density (HT-LS), or (4) high tilapia and high shrimp density (HT-HS), and 2 scenarios with average stocking density and (5) low feeding rate (LFR), or (6) high feeding rate (HFR).

## Results

In the stocking density scenarios, higher tilapia stocking density led to lower tilapia harvest weights, regardless of shrimp density (Table 2). For shrimp, higher tilapia density or lower shrimp density increased harvest weights due to greater feed availability. Experts validated tilapia harvest weights as realistic across all scenarios but noted that shrimp harvest weights in LT-HS and HT-HS appear to be underestimated. Higher stocking density raised the cumulative mortality of the same species. Higher shrimp densities slightly reduced tilapia mortality (Table 2). But higher tilapia density slightly increased shrimp mortality. Experts remarked that tilapia cumulative mortality appeared to be overestimated at high densities, while shrimp mortality was within a realistic ranges.

Under the HFR scenario, tilapia growth was 2.7 times faster to a harvest weight of 205 grams (Table 2), and shrimp harvest weights were 3.8 times higher than under LFR. Experts agreed with the faster tilapia growth at higher feeding rates but pointed out that shrimp harvest weights under LFR appear to be underestimated. Operating profit under HFR was USD 1.54 higher than under LFR (Table 2), though experts cautioned that this large difference may indicate model overestimation.

## Discussion

The lower than expected shrimp harvest weights can be attributed to a lower realized feeding rate from uneaten tilapia feed, a low and constant natural feed availability assumed by the model, and potentially unrealistic estimates for the effect of stocking density and FCR used to calculate economic FCR. Experts expected tilapia cumulative mortality of around 30% in the HT-LS and HT-HS scenarios. The discrepancy arises because the daily mortality model links higher biomass to higher daily mortality rates. Experts also noted that mortality is typically higher in the early weeks after stocking for both species due to biological vulnerability. The annual operating profit projected under the HFR scenario appears high. The model focuses on the effects

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Table 1. Input for stocking density (SD) and feeding rate (FR) scenarios

| Input | SD scenarios | | | | FR scenarios | |
|---|---|---|---|---|---|---|
| | LT-LS | LT-HS | HT-LS | HT-HS | LFR | HFR |
| Duration (day) | 120 | 120 | 120 | 120 | 240 | 90 |
| Tilapia stocking density (fish/$m^2$) | 1 | 1 | 5 | 5 | 3 | 3 |
| Tilapia stocking weight (g) | 10 | 10 | 10 | 10 | 10 | 10 |
| Shrimp stocking density (shrimp/$m^2$) | 5 | 15 | 5 | 15 | 10 | 10 |
| Shrimp stocking weight (g) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Feeding rate (% biomass) | 2 | 2 | 2 | 2 | 1 | 3 |

LT: Low tilapia, LS: Low shrimp, HT: High tilapia, HS: High shrimp, LFR: Low feeding rate, HFR: High feeding rate.

Table 2. Main outputs for stocking density (SD) and feeding rate (FR) scenarios

| Output | SD scenario | | | | FR scenario | |
|---|---|---|---|---|---|---|
| | LT-LS | LT-HS | HT-LS | HT-HS | LFR | HFR |
| Rounds (per year) | 2.6 | 2.6 | 2.6 | 2.6 | 1.3 | 3.4 |
| Tilapia harvest weight (g) | 193.0 | 192.9 | 161.0 | 160.8 | 204.6 | 204.7 |
| Tilapia cumulative mortality (%) | 15.8 | 14.8 | 45.2 | 44.9 | 55.7 | 29.1 |
| Shrimp harvest weight (g) | 4.4 | 1.6 | 14.0 | 4.6 | 3.1 | 11.8 |
| Shrimp cumulative mortality (%) | 32.9 | 34.1 | 38.6 | 39.2 | 54.5 | 35.0 |
| Operating profit (USD/$m^2$/year) | 0.14 | 0.04 | 0.55 | 0.25 | 0.11 | 1.65 |
| Gross revenue (USD/$m^2$/$year^1$) | 0.63 | 0.64 | 1.72 | 1.53 | 0.57 | 3.11 |
| Operating cost (USD/$m^2$/$year^1$) | 0.49 | 0.59 | 1.18 | 1.28 | 0.46 | 1.46 |

LT: Low tilapia, LS: Low shrimp, HT: High tilapia, HS: High shrimp, LFR: Low feeding rate, HFR: High feeding rate.

of species interactions on the total economic performance, and does not account for the effects of disease and other risks which will likely reduce the annual profit when they affect the pond. The main aim for the model is to assess the effects of management choices on the technical and economic performance. Availability of the data on the performance of polyculture ponds is limited which, when available, will allow finetuning of the model parameters, especially for growth on natural feed and for the interaction effects on mortality.

**Conclusions**

The model performs well to predict the effects of management choices on tilapia growth. Shrimp growth appears to be underestimated in some scenarios, especially with low feeding rate. Projections of mortality appear realistic in most cases, but mortality under high tilapia stocking densities tends to be overestimated. Currently, the model could serve as a tool for assessing the tilapia-shrimp polyculture, supporting food production and economic stability for smallholder farmers.

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# THE FIRST STUDY ON THE EFFECT OF DIETARY BLACK SOLDIER FLY (*Hermetia illucens*) PRE-PUPAE MEAL ON FLATHEAD GREY MULLET (*Mugil cephalus*) GROWTH, GUT HEALTH AND TRANSCRIPTOMIC PROFILE

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Developing sustainable aquafeeds and diversifying farmed fish species, with focus on that with a low dependency on marine-derived ingredients, is crucial for the modern aquaculture. In the present study, for the first time, low-fish meal diets, in which black soldier fly (*Hermetia illucens*) meal was used to partially replace conventional ingredients, were formulated to assess the effect on growth, gut health and transcriptomic profile of flathead grey mullet (*Mugil cephalus*). Four, iso-proteic, iso-lipidic and iso-energetic diets were formulated to replace the conventional protein sources of a control diet (H0) with 10, 15 and 20 % black soldier fly (*Hermetia illucens*) meal (H10, H15 and H20, respectively). For the feeding trial, 360 juvenile grey mullets (weight = 40.2±0.5 g), obtained by the International Marine Center (IMC, Oristano, IT), were transferred to the Institute of Marine Biological Resources and Biotechnologies, National Research Council (IRBIM-CNR, Messina, IT). After acclimatation, fish were randomly divided in 12 experimental tanks, connected to an open recirculating system located on the Sicilian coast of the Strait of Messina (Temperature, 22,05±9,12 °C; $O_2$, 66.5±8.1 mg $L^{-1}$; Salinity=, 39.07±0.41 PSU; pH, 8.62±0.42). Fish were assigned to the four dietary treatments, in triplicate and fed the experimental diets (1-3% body weight, according to the water temperature) for 138 days. At the end of the experiment, after euthanasia (MS-222 500 mg $L^{-1}$), fish were individually weighted and gut, liver and spleen were sampled and processed for histological and immunohistochemical analyses.

At the end of the experiment, fish fed H15 and H20 diets showed a significantly lower final weight compared to those fed H0 and H10 diets. Moreover, fish fed diet H20 showed a significantly lower Fulton condition factor compared to the other groups. Gut histology was marginally affected by the experimental diets (fig.1 a); however, a significant increase in villi thickness and mucous cells abundance was evidenced in group H15. No alterations in liver and spleen histology were observed within the experimental groups. Transcriptomic analyses of intestine revealed changes in expression of genes involved in inflammation, cell recognition patterns and lipid uptake in response to *Hermetia illucens* meal inclusion. According to the results obtained in the present study, the tolerance of *M. cephalus* to dietary *H. illucens* meal is lower compared to other, strictly carnivorous farmed fish species. However, replacing conventional protein sources with 10% *H. illucens* meal can play a role in improving fish gut health as also suggested for the first time in this species by the transcriptomic analyses, suggesting a role of this ingredient in improving fish immune defenses, without affecting fish zootechnical performances.

**Founding:** This project was funded by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 Call for tender No. 3138 of 16 December, 2021, rectified by Decree n.3175 of 18 December, 2021, of Italian Ministry of University and Research funded by the European Union–NextGenerationEU; award number: project code CN_00000033, Concession Decree No. 1034 of 17 June, 2022, adopted by the Italian Ministry of University and Research, CUPB83C22002930006, Project title “National Biodiversity Future Center-NBFC.”

# DIETARY ADMINISTRATION OF THE HOST DEFENSE PEPTIDE PISCIDIN 1: IMMUNE AND TEXTURAL EFFECTS IN GILTHEAD SEABREAM (*Sparus aurata*)

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**Introduction**
Host defense peptides (HDPs) have garnered significant attention as innovative alternatives to conventional antibiotics. These peptides are notable not only for their antimicrobial properties but also for their capacity to modulate immune responses. Piscidins are amphiphilic, cationic peptides characterized by an α-helical secondary structure, potent antimicrobial activity against a broad spectrum of pathogens, and notable immunomodulatory properties [1]. However, their oral administration is constrained by limited stability and vulnerability to enzymatic degradation [2].

**Methodology**
To address these limitations, we encapsulated piscidin 1 (P1) within carrageenan and pectin microparticles, thereby developing an oral delivery system. P1 is an HDP of gilthead seabream (*Sparus aurata)*. Gilthead seabream specimens were fed for 17 days a commercial diet (Skretting) supplemented with 0%, 0.05%, or 0.1% encapsulated P1. The scanning electron microscope was used to determine the size of the microparticles. Serum and head-kidney leucocytes were collected on days 4 and 17 to assess humoral and innate immune parameters (peroxidase, anti-protease, lysozyme, and respiratory burst activities and phagocytosis). Additionally, muscle samples were collected on day 17 for texture profile analysis (TPA; hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience) and proximate composition analysis (carbohydrates, protein, fat, ash, fiber, water).

**Results**
The results indicated that serum peroxidase activity significantly increased on day 4 in fish fed the 0.05% encapsulated P1 diet. Respiratory burst activity decreased in fish fed with encapsulated P1 at both day 4 and day 17. Phagocytic ability increased in fish fed the 0.1% diet at both sampling points, while only the 0.05% diet group exhibited an increase in phagocytosis capability on day 4. TPA results demonstrated that adhesiveness decreased in fish fed the 0.1% encapsulated P1 diet, while resilience increased in both groups receiving encapsulated P1. The diet had no effect on the proximate composition of the fish.

**Conclusion**
Therefore, this study suggests that encapsulated peptides may hold potential as an oral delivery system to stimulate the immune system and enhance muscle quality in aquaculture.

**Acknowledgments**
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# FATTY ACID COMPOSITION OF AMPHIPODS IN BRACKISH AND MARINE ENVIRONMENTS

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## Introduction

Salinity gradients play a crucial role in coastal ecosystems by supporting biodiversity, regulating nutrient cycling, and contributing to overall ecosystem resilience. Marine organisms depend on specific salinity ranges for optimal physiological function, and deviations from these ranges can impair organism health and trigger cascading effects on population dynamics and ecosystem stability. Understanding how salinity variation influences marine organisms and how it relates to biological features such as sex is essential, as it directly impacts key cellular processes, such as lipid metabolism and the structural integrity of cell membranes. Assessing the factors that shape the lipid composition of marine organisms is key for aquaculture species, as optimizing lipid profiles can enhance their nutritional value and improve survival rates during early life stages. At a production level, such insights can improve species selection, refine rearing protocols, and support effective water quality management. In this study, we investigated whether the fatty acid (FA) composition of two gammarid amphipod species, *Melita palmata* and *Gammarus locusta*, differs between males and females, as well as in response to salinity variations along a natural gradient in Ria de Aveiro coastal lagoon (Portugal). Special emphasis was placed on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), given their essential roles in cellular homeostasis and nutritional value for marine species.

## Materials and methods

Three sampling sites were selected along Mira channel (Ria de Aveiro, Portugal), encompassing a natural salinity gradient from marine to freshwater conditions: S35 (~35 salinity), S30 (~30 salinity), and S3 (~3 salinity). Fatty acid methyl esters were individually extracted from *M. palmata* and *G. locusta* adult amphipods using gas chromatography-mass spectrometry (GC-MS). The overall abundance of the most important FA (measured as μg of FA per mg of amphipod dry weight), including EPA and DHA, was compared separately for each species between salinities and sexes using a multivariate approach. Differences in the abundance of each FA between the same factors were assessed through univariate linear models.

## Results

The overall FA composition of *M. palmata* varied significantly with both salinity and sex (Fig. 1a). Regardless of the salinity level, females showed a higher FA abundance than males (females: 14.3 $\mu g.mg^{-1}$; males: 11.1 $\mu g.mg^{-1}$). Additionally, amphipods from the high-salinity site exhibited greater total FA content compared to those from the low-salinity site (S30: 14.6 $\mu g.mg^{-1}$; S3: 10.8 $\mu g.mg^{-1}$). In contrast, the overall FA composition of *G. locusta* differed according to sex (females: 17.2 $\mu g.mg^{-1}$; males: 7.5 $\mu g.mg^{-1}$) but not between salinity levels (S35: 24.45 $\mu g.mg^{-1}$; S30: 24.85 $\mu g.mg^{-1}$), with higher content for females than males (Fig. 1b). Salinity gradients significantly influenced the FA composition of *M. palmata*, with high-salinity conditions promoting the accumulation of EPA and DHA, regardless of sex. In contrast, while salinity had no measurable effect on the FA composition of *G. locusta*, females consistently exhibited nearly three times higher levels of EPA and DHA, when compared to males.

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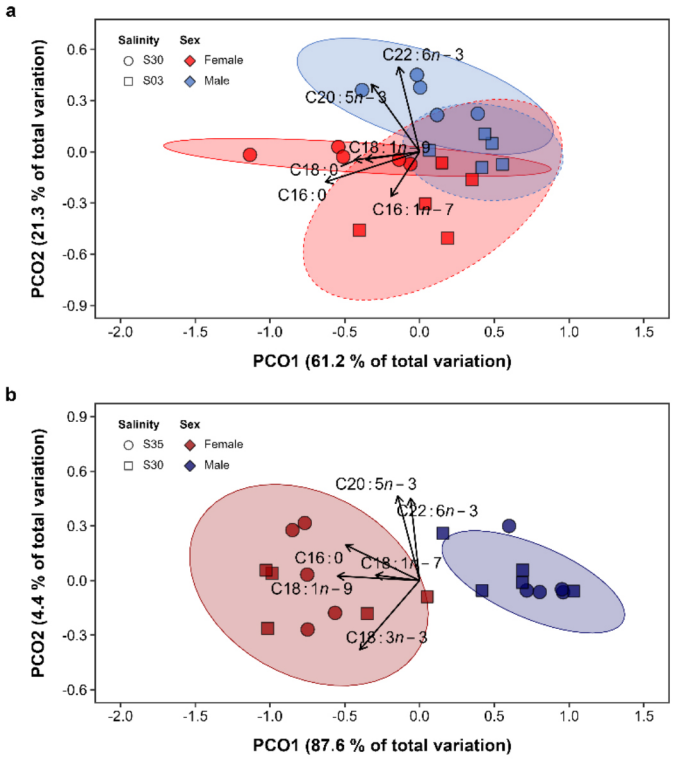


**Figure 1.** Principal Coordinate Analysis (PCO) illustrating the variation of the fatty acid (FA) composition of the amphipods a) *Melita palmata* and b) *Gammarus locusta* according to different salinity levels (Salinity 35 - S35; Salinity 30 – S30; Salinity 3 – S3) and the sex (female and male) of the individuals. Analyses were restricted to FA accounting for more than >1% of total FA abundance. Vector loadings were represented only by the FAs that contributed the most to the differences between factor levels.

## Discussion

The observed differences in FA composition suggest that each species employs distinct strategies to cope with environmental salinity stress. In both gammarid amphipod species, females exhibited higher FA content than males, likely reflecting the greater energy demands associated with reproduction. *Melita palmata* showed high plasticity along the salinity gradient, with significant variation in FA composition being linked to both salinity and sex. This indicates that *M. palmata* is well-adapted to dynamic estuarine conditions and may possess a flexible metabolic strategy beneficial for survival and potentially valuable for aquaculture in environments with variable salinity. In contrast, *G. locusta* exhibited a strong sex-related difference in FA composition, but no significant response to salinity, probably due to the limited salinity variation between the sampled locations. Nevertheless, these results suggest that in aquaculture settings, favouring an all-female population, whether through selective rearing or sex-inducing technologies, can enhance biomass yield and profitability, owing to females' superior FA composition.

## Conclusion

Salinity- and sex-specific patterns in *M. palmata* and *G. locusta* revealed that salinity conditions can shape the FA composition of each species, affecting males and females differently. These patterns highlight the species' capacity to tolerate moderate salinity fluctuations, reflecting their adaptability to dynamic coastal environments. Beyond advancing our understanding of salinity-driven physiological adaptations in marine and estuarine amphipods, this study offers valuable insights for improving aquaculture practices. By examining how salinity influences FA composition, particularly the levels of EPA and DHA, our findings can support more informed decisions on species selection and salinity management to enhance the nutritional value of cultured organisms.

## Acknowledgements

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# OPTIMISING VITAMIN E PRODUCTION IN THE MICROALGA *Phaeodactylum tricornutum* FOR SUSTAINABLE AQUAFEED FORMULATIONS

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## Introduction

Microalgae are increasingly recognised as a promising resource for sustainable aquaculture, not only due to their protein and lipid profiles but also because of their richness in bioactive compounds with antioxidant proprieties (Dineshbabu et al., 2019). Among these, vitamins such as C and E play a significant role in maintaining cellular homeostasis and mitigating oxidative stress in aquatic organisms. The presence and concentration of these compounds can vary significantly depending on the microalgal species, cultivation parameters, and biomass processing techniques (Goiris et al., 2012; Mavrommatis et al., 2023). As such, the strategic cultivation and harvesting of microalgae have become an area of interest for enhancing their functional value in supporting reproductive health and performance in aquaculture species (Shastak and Pelletier, 2023).

Vitamin E (α-tocopherol) is a fat-soluble antioxidant essential for the health and reproductive performance of farmed fish. It contributes to membrane stability, supports reproductive function, particularly by protecting gametes from oxidative stress and aids immune response, making it a critical micronutrient in aquafeed formulations aimed at enhancing breeding success. While traditionally supplied in synthetic form, there is growing demand for natural, sustainable alternatives. *Phaeodactylum tricornutum*, a model marine diatom, stands out due to its established cultivation protocols, rapid growth, and genetic tractability. Recent efforts have focused on optimising culture conditions to stimulate the biosynthetic pathways responsible for vitamin E production in this species. Increasing the endogenous levels of α-tocopherol within *P. tricornutum* biomass could reduce the total quantity of microalgae required to achieve the desired reproductive support in aquaculture broodstock diets. This biofortification approach offers multiple benefits, including lower production costs, enhanced sustainability, and reduced reliance on external synthetic inputs, aligning with current trends in the development of environmentally responsible aquaculture systems.

## Materials and Methods

This study evaluated the effects of exogenous vitamin supplementation on the growth and antioxidant potential of the marine diatom *Phaeodactylum tricornutum*. Cultures were obtained from GreenCoLab (University of Algarve) and maintained in a sterile 800 mL photobioreactor containing either standard algal growth medium (control) or medium supplemented with α-tocopherol acetate (vitamin E) in concentrations ranging from 2 to 10 mg·L$^{-1}$. The vitamin solution was sterilised via 0.22 µm Millipore membrane filtration before use. Cultures were incubated in triplicate for each treatment for 10 days at 25 ± 2 °C under white light illumination (2,500 lux) and constant aeration. Biomass was harvested at predefined time points, freeze-dried, and stored until further analysis. Vitamin content was quantified using chromatographic techniques to assess the intracellular accumulation of supplemented compounds. Additional parameters such as culture density and biomass yield were also monitored throughout the experiment.

## Results and Discussion

Specific supplementation strategies with α-tocopherol are expected to enhance intracellular vitamin E accumulation in *Phaeodactylum tricornutum* without compromising biomass productivity, offering an effective means of producing antioxidant-enriched microalgal biomass for targeted applications. In the present study, α-tocopherol acetate supplementation at 2, 4, 6, 8, and 10 mg·L$^{-1}$ yielded vitamin E contents of 36.85, 96.62, 171.99, 64.25, and 38.51 mg per 100 g DW of sample, respectively, compared with only 8.88 mg per 100 g DW in the control, clearly demonstrating a concentration-dependent response. Cultures grown under optimised conditions thus exhibited markedly improved antioxidant profiles, with the highest intracellular vitamin E accumulation observed at 6 mg·L$^{-1}$, suggesting this concentration as a potential optimum for enrichment purposes, whereas excessive supplementation (≥8 mg·L$^{-1}$) led to a pronounced decline in vitamin E content, possibly due to metabolic regulation or feedback inhibition mechanisms.

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The production of vitamin E-enriched *P. tricornutum* biomass represents a promising strategy to improve male reproductive performance in fish. Vitamin E plays a critical role in protecting spermatozoa from oxidative damage, preserving membrane integrity, motility, and fertilisation capacity. By reducing oxidative stress during spermatogenesis, particularly under periods of high metabolic demand, α-tocopherol-rich microalgal supplementation may enhance sperm quality parameters and increase successful fertilisation rates. These findings underline the potential of targeted vitamin E enrichment in microalgal biomass as a functional approach to boosting broodstock reproductive efficiency in aquaculture breeding programs.

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# TOWARDS SUSTAINABLE MUSSEL FARMING: INNOVATIVE PRACTICES AND PERCEPTIONS OF ITALIAN OPERATORS

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## Introduction and background

The Strategic guidelines for a more sustainable and competitive European aquaculture sector address challenges and opportunities in line with the European Green Deal and related strategies. A key priority is to reduce the environmental impact of aquaculture products by promoting the use of more sustainable technical and biological inputs under the call of the circular economy (COM, 2019).

Mussel farming, one of the most important sub-sectors in Europe, is facing new challenges as it has been identified as a significant source of marine pollution (Tudini & Forgione, 2024). Indeed, the sector extensively uses plastic materials for production and packaging (Fortibuoni et al., 2021; Bottari et al., 2024). It is estimated that approximately 20 grams of plastic are used for each kilogram of mussels marketed (Pietrelli, 2022).

As part of the outcomes of the "LIFE MUSCLES" project (LIFE20 ENV/IT/000570), this study aims to investigate the economic viability of using biodegradable or recycled nets in Italian mussel farming. Specific objectives include (i) identifying the main barriers to adoption, (ii) exploring the perspectives of Italian mussel farmers, and (iii) offering policy recommendations in line with European strategies.

## Methodology

Interviews with Italian mussel farmers were conducted to identify the main cost factors of sustainable alternatives to conventional nets. A contingent valuation model (Carson, 2005) was used to estimate willingness to pay (WTP) based on (1) farmers' subjective knowledge and (2) objective information provided during the study on the characteristics of recycled and biodegradable nets. Economic data were also analyzed to assess costs and benefits.

## Results and conclusions

While mussel farmers acknowledge the environmental advantages of alternative nets, widespread adoption remains limited due to key challenges such as high initial costs, doubts about performance, lack of institutional backing, and uncertainty surrounding consumer acceptance. These findings highlight the urgent need for coordinated institutional support, including financial incentives, a robust regulatory framework, and targeted awareness campaigns.

A comprehensive policy approach - integrating funding, training, research, and consumer engagement - is essential to driving the transition toward sustainable practices in the mussel farming sector.

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Life MUSCLES: Circular innovation to protect the sea. https://lifemuscles.eu/

# DO PHOSPHOLIPIDS AND BILE SALTS SHAPE LARVAL GROWTH, SURVIVAL AND DEVELOPMENT IN ATLANTIC COD *Gadus morhua*?

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## Introduction

Atlantic cod (*Gadus morhua*) farming has recently resurged in Norway, but larval nutrition remains a major challenge (Nardi et al., 2021), and no commercial diets are specifically tailored to cod larvae. Lipids are a key dietary component, providing both energy and essential fatty acids (Hamre et al., 2013). Cod larvae are thought to have limited ability to digest dietary lipids due to low endogenous bile salt (BS) levels (Olsen et al., 1991). Adding BS to the diet could improve lipid digestion, as seen in other fish species (Wang et al., 2023). After absorption in enterocytes, lipids are transported to peripheral tissues in lipoproteins, but this process may be restricted in larvae due to low *de novo* synthesis of phospholipids (PLs) (Teshima et al., 1987), an essential component of lipoproteins. Fish larvae therefore need more dietary PL than juvenile and adult fish (Tocher et al. 2008), yet the specific requirements in cod larvae remain unclear.

This study aimed to assess how dietary PL level, BS supplementation, and their interaction (PL×BS) affect growth, survival, and development in cod larvae. We hypothesized that larval performance would be improved by (1) a higher dietary PL level and (2) BS supplementation, and (3) that the effect of BS would be more pronounced at low PL levels, where the diets contained more neutral lipids to maintain an isolipidic formulation.

## Materials and methods

Atlantic cod larvae were reared in 200 L tanks and fed one of four formulated diets from 17 to 61 days post-hatching (dph), using a 2×2 factorial design with $n = 3$ tanks per group. The diets differed in PL level – high (7 % dry matter, dm) or low (6 % dm) – and BS supplementation – 0.04 % dm or 0 % dm – yielding the following treatment groups: HPL (high PL), HPL-BS (high PL with BS), LPL (low PL), and LPL-BS (low PL with BS).

Larvae were sampled throughout the experiment to measure dry weight (DW) and standard length (SL), and for histological analysis of the liver. At 61 dph, samples were taken for bone staining to assess skeletal ossification and anomalies, as well as biochemical analyses of proximate composition, lipid classes, and fatty acid composition. Survival from 20 to 60 dph was calculated by daily counts of dead larvae.

## Results

No significant differences in larval growth (DW and SL) were observed among the groups at the end of the experiment. Nonetheless, the HPL group had significantly higher DW compared to the LPL-BS and LPL groups at 38 and 45 dph, respectively. Larvae receiving high-PL diets also showed a modest but significant increase (6 %) in specific growth rate (SGR) compared to larvae receiving low-PL diets between 15 and 52 dph. Survival at 60 dph ranged from 42 to 60 % across the treatment groups, with no significant differences.

Liver histology showed a decrease in hepatocyte nucleus size following 15 dph, with a subsequent increase towards 61 dph. Notably, LPL-BS larvae maintained significantly smaller nuclei at the end of the trial. Hepatic energy storage increased markedly between 45 and 61 dph, with a tendency for higher storage in high-PL groups (22%) compared to low-PL groups (12%) at 52 dph. Energy storage was closely associated with larval size and followed a Gompertz growth model (sigmoid curve), with no differences between the dietary treatments.

Bone staining revealed significantly more ossified dorsal and anal fins in the HPL and HPL-BS groups compared to the LPL-BS group. Additionally, larvae fed the HPL-BS diet showed a greater number of fully ossified vertebrae compared to the LPL group. Model comparisons using Akaike's Information Criterion (AIC) indicated that these differences in skeletal development were associated with dietary PL level rather than larval size. The diets showed no consistent effects on skeletal anomalies, though the frequency of kyphosis was significantly higher in the LPL group (15 %) than in the HPL group (1 %).

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No significant diet effects were observed in larval proximate and lipid class compositions. The fatty acid composition of the PL fraction also showed no differences across diets. In contrast, the level of n-3 polyunsaturated fatty acids (PUFAs) in the triacylglycerol (TAG) fraction was significantly higher among larvae fed low-PL diets (13 %) compared to high-PL diets (9 %).

**Conclusions**

Our findings suggest that increasing dietary PL levels enhances skeletal development and, to a lesser extent, supports larval growth in Atlantic cod. These benefits may be linked to more efficient utilization of n-3 PUFAs when supplied through the dietary PL fraction rather than the TAG fraction. BS supplementation did not improve larval performance, suggesting that endogenous BS levels were sufficient to ensure effective lipid digestion under the tested dietary conditions.

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# A TWO-STEP *IN VITRO* MODEL OF DIGESTION IN *Octopus vulgaris*: RELEASE OF AMINO GROUPS FROM THE MUSCLE OF DIFFERENT PREYS

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## Introduction

The recent advances in the aquaculture of the cephalopod *Octopus vulgaris* warrant further research into specific topics about the feeding, growth, and welfare of this species in captivity. *In vitro* models of digestion have been applied to terrestrial and fish species of commercial interest to explore the potential digestibility of dietary ingredients with a reduced cost in terms of time and resources (Moyano et al., 2015). However, the application of *in vitro* techniques to the digestion of cephalopods is very infrequent (Domingues et al., 2020; Hamdan et al., 2014). The present work describes the application of a two-step *in vitro* model for the digestion of different fresh prey with the enzymatic extracts of posterior salivary glands and digestive glands from the common octopus.

## Materials and methods

Protein sources comprise the freeze-dried muscle of fish (*Hippoglossus hippoglossus*), crab (*Callinectes sapidus*), or squid (*Loligo vulgaris*). Digestive crude extracts were prepared by mixing the tissue of posterior salivary glands (PSG), or the digestive gland (DG), with distilled water in a proportion of 1:10 (w/v), homogenizing the mixture, centrifuging at 12000 g for 30 min at 4 ºC, and retaining the supernatant as the source of enzymes. Total alkaline and acid protease activities of the crude extracts were measured according to the protocol of Anson (1938). One unit (U) of activity was defined as the amount of enzyme liberating 1 µmol of product per min. under the conditions described. *In vitro* digestions were conducted in 15 mL Falcon tubes at 25 ºC under shaking, and consisted of two phases: a phase with PSG extract acting at pH 8.0 for 60 min, and a second phase with DG extract acting at pH 3.0 for 120 min. Each substrate (halibut, crab, and squid muscle) was tested three times with active extracts, and another three times with inactivated (by heating at 100ºC) extracts (blank assays). Each reactor was charged with 25 mg of crude protein, and the ratios protease: protein were 5 and 25 protease units per mg protein for the first and second phases, respectively. The reaction started with the addition of the PSG extract. After 60 minutes, the pH was reduced with HCl and the DG extract was added. The reaction medium was sampled after 5, 60, 65 and 180 minutes of digestion. Samples were stored at -80ºC until being analyzed for the quantity of amino groups according to the OPA spectrophotometric method (Zahir et al., 2018). Data on the released amino acids at each sampling time (calculated as the soluble amino groups in the reactor with active extracts minus the same variable in the reactors with inactivated extracts) were analyzed using a repeated-measures ANOVA with time as the repetition variable, and prey as the among-subjects factor. Statistical significance was set at $p < 0.05$.

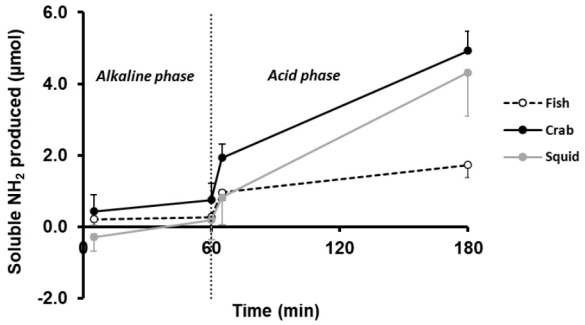


Fig. 1. Production of amino groups in a two-step *in vitro* model of the digestion of fresh muscles from fish, crab, and squid with digestive enzymes from *O. vulgaris*.

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## Results

The release of amino acids was clearly affected by time ($p < 0.001$). The production of amino acids was very low at the end of the alkaline phase, but increased rapidly after the pH was lowered to 3.0, at the beginning of the acid phase of digestion. This increase in amino acid release continued throughout the acid phase. The effect of the type of protein source on the amino acid production was marginally significant ($p = 0.062$), with higher production observed when the substrate was crab or squid muscle. The effect of the interaction term (time x prey) was highly significant ($p < 0.001$), indicating a faster release of amino acids from crab and squid muscles compared to fish muscles.

## Discussion

The *in vitro* model described herein reveals differences in the release of amino acids from different prey when digested with digestive extracts of *O. vulgaris*. Proteins from preferred prey, such as decapod crustaceans, seem to be more easily hydrolyzed by octopus' digestive extracts. The first phase of the digestion carried out with enzymes from the posterior salivary glands at an alkaline pH, was nearly ineffective to release amino groups. It is possible that this phase mainly consisted in the production of large polypeptides instead of free amino acids or small oligopeptides, as it occurs when simulating the gastric phase of digestion in fish (Grabner and Hofer, 1989). Further research is necessary to clarify this point. In contrast, Hamdan et al. (2014) reported that proteins from fish-based ingredients (fish meal) were more easily hydrolyzed by octopus digestive enzymes than proteins from a crustacean-based ingredient (krill meal). However, those ingredients were subjected to heating processes, and it is known that the thermic treatment of fresh preys depresses the assimilation of nutrients by living cephalopods (Domingues et al., 2009). The intensity of this phenomenon is not only related to the temperature of the heating process but, possibly, to the source of protein. Domingues et al., (2020), applied a one-phase *in vitro* digestion model (only the acidic phase) to digest different fresh or heated prey, measured the degree of hydrolysis (DH) by the pH-Stat method, and reported a higher DH for marine crabs and squids than for terrestrial crabs and marine fish when this prey was freeze-dried. In general, our results indicate that *in vitro* digestion models can be applied to cephalopods species but, in the future, it will be important to unify protocols and techniques to compare the results obtained by different authors.

## Acknowledgements

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# DIFFERENTIATION BETWEEN *Neobenedenia spp.* AND *Benedenia seriolae* USING A PCR-RFLP ASSAY

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## Introduction

Monogenean parasites, particularly skin ectoparasites *Neobenedenia spp.* and *Benedenia seriolae*, represent a significant threat to marine aquaculture species such as *Seriola dumerili*. Specifically, *Neobenedenia spp* has been identified in major outbreaks causing losses in both in juveniles and broodstock of *S. dumerili* in the Atlantic Sea. The broad host specificity reported for *Neobenedenia* is atypical for monogeneans, where it is considered that approximately 80% of all monogenean species only infect one host species and usually only in a single ocean (Brazenor et al., 2018). Both parasite species possess very similar morphological characteristics, which complicates their differentiation by traditional methods. Therefore, molecular tools can be very useful to differentiate them. This study aimed to distinguish *Neobenedenia spp.* from *Benedenia seriolae* through PCR amplification and restriction fragment length polymorphism (PCR-RFLP) analysis of the *cox1* gene.

## Materials and methods

Adult skin flukes were collected from cultured fish at the culture facilities of Canary Island Oceanographic Center (IEO-CSIC). Samples were collected by removing live parasites from the skin of their hosts using a scalpel blade. Parasites were fixed and stored in 70% analytical grade ethanol. Parasite DNA was isolated by proteinase K digestion followed by phenol-chloroform purification. The *cox1* gene was amplified by PCR following the protocol described by Kobayashi et al., 2018. Two independent digestion reactions were performed on the PCR products using the restriction enzymes EcoRI (recognizes G^AATTC sites) (Kobayashi et al., 2018) and RsaI (recognizes GT^AC sites) (Fig. 1). Digestion products were analyzed by agarose gel electrophoresis.

## Results

No digestion was observed with EcoRI, which is consistent with the restriction pattern of *Neobenedenia spp*. The independent digestion with RsaI produced two visible fragments (~700 bp and ~250 bp), as predicted in silico for *Neobenedenia spp*. A third smaller fragment (~70 bp) was expected but only faintly visible.

## Conclusions

The digestion profile obtained with RsaI supports the identification of the samples as *Neobenedenia spp*. The absence of EcoRI digestion is also compatible with this identification. PCR-RFLP provides a reliable method for differentiating monogenean parasites affecting aquaculture species.

## Acknowledgements

THINKINAZUL project (Spanish Ministerio de Ciencia e Innovación; European Union Next Generation EU (PRTR-C17. I1); Gobierno de Canarias). ACUICONECTA project (1/MAC/1/1.1/0123; Interreg MAC 2021–2027; European Regional Development Fund).

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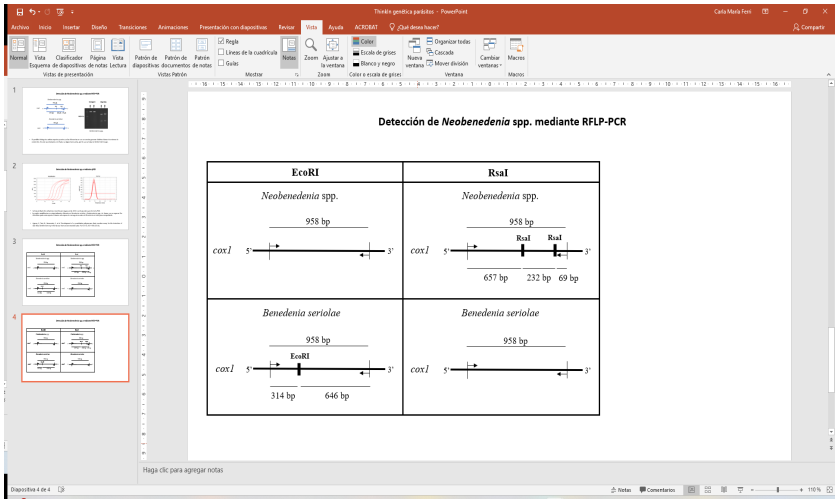


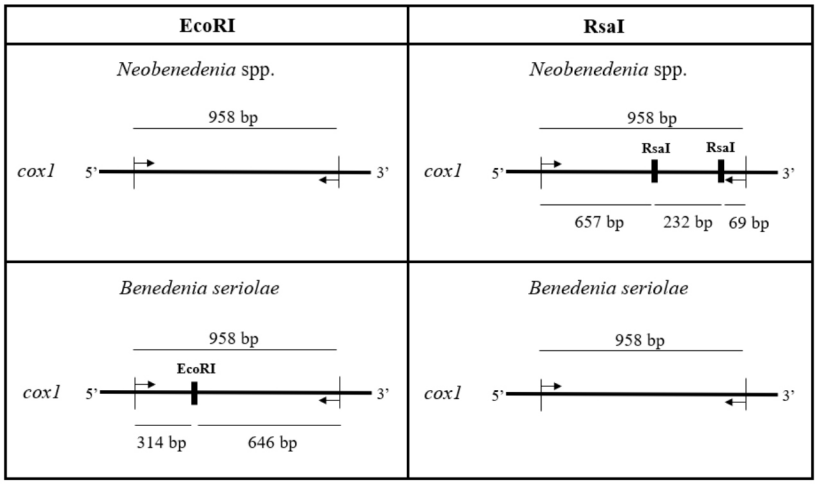


Fig. 1. Digestion patterns of *cox1* PCR products by EcoRI and RsaI in *Neobenedenia spp*. and *Benedenia seriolae*.

# EFFECTS OF A PLANT-BASED DIET DURING THE FISRT MONTH OF FEEDING ON ALEVIN RAINBOW TROUT (*Oncorhynchus mykiss*) IN THE DEVELOPMENT OF TONGUE SENSORY SYSTEM REGULATING FEEDING BEHAVIOR

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## Introduction

Taste perception is crucial for animals to detect nutrients and provide essential dietary information for growth and survival. In rainbow trout (*Oncorhynchus mykiss*) alevins, early growth performance can be influenced by food intake, particularly when fed terrestrial ingredients that exclude fish meal and fish oil. Our study aimed to investigate the role of taste receptors in nutrient detection and the associated signaling pathways leading to central nervous system activation in the regulation of feeding behavior.

## Material and Methods

We conducted a nutritional experiment from the first feeding through 30 days, comparing the performance of fish fed a commercial-like diet (C diet: a blend of fish meal, fish oil, and plant ingredients) with those on a 100% plant-based diet (V diet). After 5 and 30 days of feeding, fish were fasted for 16 hours and then re-fed with either the C or V diet, with sampling conducted at two time points: 20 minutes and 6 hours post-meal. We assessed the expression of nutrient-sensing genes related to fatty acids, amino acids, and sweetness, as well as taste receptor genes for different flavors. Additionally, we examined calcium signaling pathways in the tongue, focusing on indolamine and catecholamine pathways, alongside appetite-regulating neuropeptides in the brain and gut hormones in the intestinal system of the alevins.

## Results

Results revealed that fish on the V diet experienced a decrease in body weight gain starting from day 10, which continued through to day 30. Changes in feed intake were also observed between days 0–10 and 21–30 after the first meal. In tongue tissue, after 5 days of feeding, fish on the C diet showed a slight upregulation of nutrient taste receptors, but not those related to flavor, along with a clear activation of the calcium signaling pathway. By day 30, there was a general upregulation of both nutrient and flavor taste receptors, although calcium signaling appeared less distinct in regulation. A significant dysregulation of the serotonin pathway, along with its degradation, was observed in the tongues of fish fed the V diet at both 5 and 30 days. Notably, for the first time in fish, catecholamine quantification levels in the tongue were identified as a potential marker for nutrient detection. Fish on the V diet showed a high quantification of L-DOPA after 5 days, which was markedly reduced by day 30. This impaired turnover of monoamines and catecholamines in the tongue may disrupt the tongue-brain axis, potentially contributing to the reduced food intake observed. This hypothesis was further supported by poorly regulated brain neuropeptides and intestinal hormones in fish fed the V diet after 30 days.

## Conclusions

Together, these findings demonstrate that a plant-based diet disrupts the feeding response at an early stage, highlighting the heightened sensitivity of rainbow trout alevins' tongue sensory systems to novel food sources during critical stages of development. Our study underscores the importance of early dietary influences on taste perception and the central regulation of feeding behavior in fish.

## Acknowledgements

This research was funded by the French National Research Agency (grant no. ANR-21-CE20-0009-01 "FEEDOMEGA").

# TESTING THE EFFECTS OF ALGAL METALLOPROTEASE INHIBITORS ON GILTHEAD SEABREAM INTESTINAL INTEGRITY

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## Introduction

Matrix metalloproteinases (MMPs) are enzymes that play pivotal roles in various biological processes, including tissue remodeling and inflammatory responses across multiple organs. Certain bioactive compounds found in macroalgae—such as sulfated polysaccharides and phlorotannins—have attracted increasing attention due to their capacity to act as matrix metalloproteinase inhibitors (MMPIs), thereby modulating extracellular matrix (ECM) turnover and attenuating inflammation (Jayawardhana *et al*., 2023). In several teleost fish species, intestinal inflammation—sharing pathophysiological similarities with human inflammatory bowel disease—can be induced by diets containing plant-based ingredients (Krogdahl *et al*., 2015). However, to date, no studies have explored the potential of algal-derived MMPIs to mitigate such diet-induced enteropathies. This preliminary study aimed to: (a) determine the presence of MMPI activity in a concentrated extract obtained from the green macroalga *Ulva spp*; (b) evaluate the compound's stability under *in vitro* conditions simulating gastrointestinal digestion in *Sparus aurata* (gilthead seabream); and (c) assess its effects on intestinal function in this species when included in experimental diets.

## Materials and methods

A protein-enriched concentrated extract was obtained from the green macroalga *Ulva* spp. through a multi-step extraction process conducted at ANFACO-CECOPESCA (Vigo, Spain). The extract's potential to inhibit matrix metalloproteinases (MMPs) from *Clostridium histolyticum* was evaluated using the EnzChek™ Gelatinase/Collagenase Assay Kit (Thermo Fisher Scientific). Upon confirmation of inhibitory activity, the extract was incorporated at 12% (w/w) into an experimental diet containing 50% plant-derived ingredients, including 10% soybean-based products. This formulation was subjected to an *in vitro* digestion assay simulating seabream (*Sparus aurata*) gastrointestinal conditions, following the methodology described by Martínez-Antequera *et al.* (2023). A control diet, identical in composition but lacking the algal concentrate, was used for comparison. Both diets were subsequently administered to juvenile seabream (68.7 ± 0.1 g) over a 6-week feeding trial. At the end of the trial, fish were sampled and intestinal functionality was assessed by measuring epithelial electrical resistance and paracellular permeability using Ussing chamber techniques, following the procedure outlined by Gregorio and Fuentes (2018).

## Results and discussion

The concentrated *U. lactuca* extract exhibited strong inhibitory activity against *C. histolyticum* MMPs, achieving nearly complete inhibition (~100%) at a concentration of 50 mg/mL. This result is consistent with previously reported values for other algal products (Martínez-Antequera *et al.,* 2025). The simulated digestion of the diets demonstrated that a substantial MMP inhibitory effect was retained in the digestate following sequential exposure to gastric and intestinal enzymes (Table 1). These observations support the hypothesis that, under *in vivo* conditions, the extract may exert modulatory effects on intestinal MMP activity, potentially reflected in altered intestinal barrier functionality.

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Table 1. Inhibitory capacity on MMP of *C. hystolitycum* retained after simulated digestion of the feed including or not 12% of algal extract. Values are presented as mean ± SD for n = 3. Values not sharing a common superscript are significantly different with $p<0.05$

| **Experimental feed** | **% inhibition MMP *C. hystolyticum*** |
|---|---|
| Including *Ulva* extract | $16.21 \pm 7.48^{b}$ |
| Control | $0.00 \pm 4.33^{a}$ |

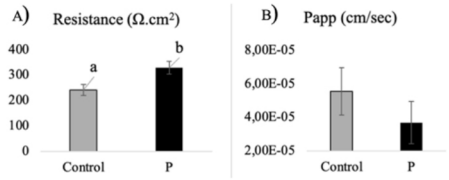


Figure 1. The effect of feeding with different experimental diets on A) Trans-epithelial electrical resistance (Rt, Ω $cm^2$) and B) Apparent permeability (Papp, cm/sec) in the anterior intestine of gilthead seabream measured *in vitro* in Ussing chambers. Values are presented as mean ± SEM. Values with $p < 0.05$ (One-way ANOVA) differ significantly.

The inclusion of the algal extract in the diet significantly increased tissue resistance (Rt) in the anterior intestine of gilthead seabream from values of 241 in the control diet to 330 Ω·$cm^2$, indicating enhanced epithelial barrier integrity. Although apparent permeability to Lucifer Yellow showed a trend toward lower values in fish fed the algal-supplemented diet, the differences were not statistically significant (Fig. 1).

The significant increase in epithelial resistance strongly suggests that bioactive compounds delivered through the algal extract played a pivotal role in enhancing intestinal tissue integrity compared to fish fed the control diet. This positive response may be attributed to either an alteration in tissue repair mechanisms or a reduction in inflammatory activity, potentially mediated by the MMP-inhibitory properties of the algal compounds. This finding may provide additional support for the incorporation of algal-derived bioactives as functional ingredients in aquaculture nutrition strategies.

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# WHERE WILL THEY SETTLE? LARVAL DRIFT MODELLING FOR OYSTER RESTORATION IN NORTHERN IRELAND

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**Introduction**

The European native oyster (*Ostrea edulis*) is a keystone species once abundant across European seas, forming extensive biogenic reefs, but it is currently considered as threatened and/or declining due to overfishing, habitat degradation, pollution, and disease (OSPAR, 2013).

Conservation and restoration efforts are now essential, not only to conserve the species but also to reinstate the ecosystem services oysters provide, such as enhancing biodiversity, improving water quality, and contributing to coastal resilience (Pogoda et al., 2020).

A critical component of effective restoration is understanding larval dispersal and recruitment, which informs the optimal placement and design of restoration sites and protected areas (Korringa, 1946). This study simulates larval dispersal and settlement potential of *O. edulis* to support targeted restoration strategies in Northern Ireland's coastal marine area.

**Modelling approach**

We applied the OpenDrift particle-tracking framework (opendrift.github.io), using its LarvalFish module to simulate the dispersion of native oyster larvae in three Northern Ireland's sea loughs: Belfast, Larne and Carlingford. Opendrift benefits from being highly customizable in terms of larval behaviour, agnostic with regard to the underlying hydrodynamic model and from allowing for a cross-domain/cross-model calculation of dispersion. It also allows for backward tracing of the dispersion paths, the introduction of pseudo-random stochastic variability and for a customizable coastline detection behaviour.

A Delft3D-Flow hydrodynamic model with a domain covering from the Larne to Carlingford Lough was used as the main source of time-varying 3-dimensional velocity fields for the Opendrift model.

A dedicated *O. edulis* larvae dispersion model was developed for the Opendrift platform (OedulisLarvaeDrift) that implements the current state-of-the-art knowledge on the early life-cycle of the native oysters. In addition to the physical behaviour of neutrally-buoyant particle, the following high-level responses were included to simulate the growth, motility, mortality and settling of the *O. edulis* larvae:

1. Time-dependent growth rates that increase the diameter of the larvae from its size at swarming time to its maximum know size before settling;
2. Size-dependent stage switching between the umbo, veliger and pediveliger stages
3. Stage-dependent vertical swimming behaviour
4. Prescribable stochastic mortality
5. Mortality due to environmental limits (salinity and temperature)
6. Stage maturity for settling, ensuring that only larvae at the pediveliger stage settles
7. Settling behaviour, including seafloor detection and viable habitat detection that ensures the settling of only the matured larvae that reach the bottom at a viable habitat
8. Exclusion of the settled larvae from the calculation to prevent their aging, growing, mortality and movement.

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As the availability of suitable settlement substrate is one of the main factors governing recruitment success of oyster populations (Rodriguez-Perez et al. 2021), our dispersal model integrates benthic habitat maps to evaluate the settlement success in the three loughs.

Larval sampling was conducted early in the seedling collection season to calibrate the horizontal distribution of hatched larvae to be used as the initial distribution for particle tracking. A sensitivity analysis explored how variation in uncertain parameters—such as pediveliger stage duration, swimming speed, and survival rates—affected dispersal and settlement outcomes.

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# INTEGRATING IMASHRIMP, A DEEP LEARNING AND COMPUTER VISION TOOL FOR MORPHOMETRIC ANALYSIS, INTO COMMERCIAL-SCALE SHRIMP BREEDING PROGRAMMES

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## Introduction

The implementation of genetic breeding programmes (GBPs) in the *Penaeus vannamei* production sector has reduced generation intervals and demonstrated significant genetic gains for traits related to growth and survival (Castillo-Juárez *et al.,* 2015; Martínez Soler *et al.,* 2024). External morphometric traits—reflecting the species' health status under specific culture conditions (Singh *et al.,* 2017)—have emerged as valuable alternative to traditional body weight measurements for improving shrimp performance. These traits exhibit high heritability ($h^2$), strong positive genetic correlations ($r_g$) with both weight and product quality traits and offer high measurement precision (Shin *et al.,* 2023; Martínez Soler *et al.,* 2024). Moreover, they can be measured non-invasively through image analysis technologies. The objective of the present study is to integrate the IMASHRIMP system—which combines deep learning and computer vision—as an advanced tool to enhance genetic selection of *P. vannamei* within the PMG-BIOGEMAR© GBP at commercial scale.

## Materials and methods

588 shrimp from the sixth generation ($G_6$) of the PMG-BIOGEMAR© GBP—genetically selected for growth traits and reared under commercial conditions—were photographed at harvest size using an Intel® RealSense™ D435 depth camera. Each shrimp was imaged from both lateral (right and left) and dorsal views, with four different angles captured per view, resulting in twelve images per individual. The initial module of the IMASHRIMP system uses pose estimation algorithms to automatically identify the shrimp's orientation (lateral or dorsal view; right or left side) and detect the presence or absence of the rostrum. Based on this information, the system applies one of two customized skeletal key point models—depending on the detected view and rostrum presence—to extract morphometric traits in pixels. A second module then converts the pixel-based measurements into centimetres using a Support Vector Machine (SVM) regression model. The resulting traits are classified as non-invasive morphometric traits (mNiT). To validate the IMASHRIMP system, the 588 shrimp were manually measured for the same 22 morphometric traits—designated as invasive morphometric traits (miT)—as well as for body weight. These traits, related to length, width, and height, were previously defined and validated by Shin *et al.* (2023) and Martínez Soler *et al.* (2024). All shrimp were genotyped using the AQUAarray LD™ SNP-chip at the Center for Aquaculture Technologies (California, USA). Additive genetic components for all morphometric traits were estimated using the Restricted Maximum Likelihood (REML) method. Best Linear Unbiased Prediction (BLUP) evaluation was performed using VCE (version 6.0) and supporting software.

## Results

At 103 days after hatching (DAH), the weight and total length (TL) of shrimp were 23.74 g and 14.35 cm, respectively, following five generations of genetic selection. Shrimp analysed were assigned to 135 full-sib and 25 half-sib families. $h^2$ for weight was 0.37. For miT, $h^2$ values ranged from 0.05 to 0.40 for length traits, while for mNiT, they ranged from 0.14 to 0.36. The lowest $h^2$ was observed for first segment length (SL1), and the highest for cephalothorax length (CL) and sixth segment length (SL6) in both miT and mNiT. For height traits, $h^2$ ranged from 0.13 to 0.30 for miT and from 0.21 to 0.41 for mNiT. When shrimp were measured using IMASHRIMP system from a lateral view, all morphometric traits—except CL—showed

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increased $h^2$. The average increase for length traits was 90%, while the average increase for height traits was 37.40%. When mNiT measurements were taken from a superior view, $h^2$ values for length ranged from 0.13 to 0.45, with the highest value found for abdominal length (AL). In this case, $h^2$ increased by an average of 95% compared to miT. Regarding width traits measured from a superior view, $h^2$ ranged from 0.08 to 0.40 for miT and from 0.10 to 0.33 for mNiT. $r_g$ between miT and mNiT ranged from 0.81 to 1.00 for lengths (lateral view in mNiT), from 0.53 to 1.00 for heights, from 0.72 to 0.98 for lengths (superior view in mNiT), and from 0.98 to 1.00 for widths. $r_g$ between mNiT and weight ranged from 0.82 to 1.00.

**Discussion**

Shrimp from $G_6$ exhibited 30% higher body weight 27 days earlier in commercial estuaries compared to second generation ($G_2$) (Shin *et al.,* 2023), demonstrating that genetic selection is significantly accelerating growth under farming conditions. However, increases in length were less pronounced (8%) compared to width (15%) and height (16%). The inclusion of morphometric traits as selection criteria is particularly relevant for shrimp markets in the USA and Europe, where whole animals are marketed and consumers place high value on exoskeletal integrity. But comprehensive morphometric assessments in animal production are typically labour-intensive, time-consuming, and costly. Recent advances in deep learning and computer vision (Chai *et al.,* 2021) offer new opportunities for aquaculture by enabling high-throughput, cost-effective measurement of traits that were previously difficult to assess. The IMASHRIMP system represents a robust technological tool for integration into the sampling workflows of shrimp GBPs. To the best of our knowledge, this is the first application of pose estimation in the shrimp aquaculture sector. IMASHRIMP significantly reduces sampling time, minimizes measurement error, and increases the $h^2$ of key morphological traits in *P. vannamei*. Successfully integrated into the PMG-BIOGEMAR© GBP, this system enhances selection accuracy and supports long-term genetic improvement.

**Acknowledgments**

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# EFFECTS OF SEASONAL TEMPERATURE CHANGES AND PRE-SLAUGHTER FASTING ON STRESS RESPONSE AND MEAT QUALITY IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

In aquaculture, fasting is a management strategy implemented before routine operations to optimize efficiency, enhance flesh quality and support animal welfare. However, its benefits depend on the duration of food deprivation and environmental conditions, as fasting is influenced by water temperature and, therefore, the increasing impact of global warming on aquatic environments. Studies suggest that fasting for approximately 55-58 degree days (°C d) minimizes the impact on stress response while enhancing muscle structure, biochemical processes and overall quality. However, seasonal temperature variations lead to shorter fasting periods in summer and longer ones in winter. Given that rainbow trout are a cold-water species with an optimal temperature range of 12 to 18°C, analyzing the effects of fasting across different temperature would be particularly valuable. This is especially crucial in light of the increasing risk of heat stress in rainbow trout farming due to global warming.

## Material and methods

A total of 495 rainbow trout were distributed across nine cages with an average water temperature of 22.0 ± 0.06 °C in summer and 8.8 ± 1.79 °C in winter. After a two-week acclimation period with daily feeding, fish were subjected to different pre-slaughter fasting durations: no fasting (0D), 3 days (65.49 ± 0.22°C d) in summer and 6 days (58.7 ± 2.21°C d) in winter (50D), 6 days (131.34 ± 0.07°C d) in summer and 13 days (114.5 ± 1.86°C d) in winter (100D). Blood samples were collected to analyze plasma cortisol levels, liver and muscle samples were taken to assess glycogen concentration. Additionally, muscle samples were analyzed for pH, *rigor mortis* and acetylcholinesterase activity. The color of the liver and skin was also measured using the CIELAB system.

## Results and discussion

In winter, plasma cortisol levels were higher in the 50D group compared to 0D, likely due to the acute stress response triggered by sudden food deprivation. However, cortisol levels in the 100D and 0D groups remained similar across both seasons, which could indicate metabolic adaptation or hypothalamic-pituitary-interrenal axis exhaustion. Additionally, higher cortisol levels in the 50D winter group compared to 50D summer suggest that prior acclimation to elevated temperatures might limit stress responses to additional stressors. Hepatic glycogen levels were lower in summer than in winter, where a progressive decline was observed with prolonged fasting. The 100D group in winter showed values comparable to the 0D summer group, likely due to glycogenolysis maintaining blood glucose as an energy source. Muscle glycogen levels remained relatively stable across fasting durations, but they were higher in summer, possibly due to reduced swimming activity in warmer water. Glycogen directly affects post-mortem pH, with a significant negative correlation between glycogen levels and pH decline, influencing *rigor mortis*. In summer, higher glycogen levels led to greater lactic acid production and a less pronounced pH drop compared to winter. Fasted fish in winter showed higher pH, possibly due to increased pre-slaughter activity. Higher temperatures generally accelerate *rigor mortis* due to faster pH decline, but in this study, summer trout exhibited lower *rigor* at 0 h, suggesting additional influencing factors. During winter, AChE activity increased significantly with fasting duration, suggesting that AChE levels rise in catabolic tissues during prolonged starvation, contributing to muscle degradation and heightened food-seeking behavior. Unfasted fish in summer exhibited higher AChE activity than those in winter, remained elevated across fasting groups. This could be due to greater sensitivity to temperature stress. Regarding skin color, no significant variations were

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**Table 1**. Means (±SEM[1]) of plasma cortisol, hepati cand muscle glycogen (Glyc), pH, *rigor mortis*, heat shock protein 70 (HSP70) gene expression and color parameters (lightness (L*), chroma (C*), red index (a*), yellow index (b*) and hue angle (h* (º)) of liver and skin.

| | 0D | | 50D | | 100D | | Significación (p) | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | W | S | W | S | W | Se | F | Se x F |
| Cortisol (µg/dl) | 1.51 ± 1.39 | 0.71 ± 0.51 | 1.30 ± 1.03 | 3.22 ± 2.37 | 2.37 ± 1.41 | 1.43 ± 1.14 | 0.80 | <0.001 | <0.001 |
| Glyc liver (mg/g) | 55.50 ± 30.34$^{c}$ | 186.97 ± 76.53$^{a}$ | 28.74 ± 17.33$^{c}$ | 114.17 ± 52.49$^{b}$ | 29.33 ± 12.81$^{c}$ | 59.83 ± 25.96$^{c}$ | <0.001 | <0.001 | <0.001 |
| Glyc muscle (mg/g) | 12.05 ± 5.19$^{*}$ | 2.78 ± 0.96 | 10.32 ± 5.38$^{*}$ | 2.68 ± 1.39 | 11.54 ± 5.36$^{*}$ | 2.13 ± 1.49 | <0.001 | 0.442 | 0.399 |
| pH | 6.60 ± 0.10 | 6.97 ± 0.17$^{*}$ | 6.70 ± 0.13 | 6.98 ± 0.16$^{*}$ | 6.68 ± 0.09 | 6.98 ± 0.14$^{*}$ | <0.001 | 0.098 | 0.235 |
| RM | 29.74 ± 3.66$^{b}$ | 58.89 ± 5.05$^{a}$ | 24.37 ± 4.28$^{c}$ | 58.63 ± 2.72$^{a}$ | 25.41 ± 3.81$^{c}$ | 56.24 ± 3.20$^{a}$ | <0.001 | 0.003 | 0.044 |
| AChE mU/mg | 0.14 ± 0.18$^{b}$ | 0.79 ± 0.30$^{c}$ | 0.73 ± 0.29$^{b}$ | 0.95±0.19$^{ab}$ | 1.03 ± 0.36$^{a}$ | 1.11 ± 0.30$^{a}$ | 0.048 | <0.001 | <0.001 |
| L* skin | 41.02 ± 7.27$^{a}$ | 41.60 ± 9.00$^{a}$ | 41.91 ± 1.07$^{a}$ | 42.04 ± 7.83$^{a}$ | 41.96 ± 0.96$^{a}$ | 36.06 ± 5.45$^{b}$ | 0.14 | 0.10 | 0.045 |
| L* liver | 26.79 ± 2.04 | 38.04 ± 7.52 | 25.19 ± 2.04 | 37.45 ± 7.72 | 26.02 ± 2.10 | 36.27 ± 1.80 | <0.001 | 0.31 | 0.54 |
| a* liver | 9.91 ± 1.96 | 8.33 ± 2.37 | 10.12 ± 2.19 | 9.50 ± 3.48 | 10.22 ± 1.85 | 8.81 ± 1.92 | 0.001 | 0.29 | 0.51 |
| b* liver | 3.82 ± 1.65$^{d}$ | 5.96 ± 2.15$^{c}$ | 3.48 ± 1.50$^{d}$ | 7.45 ± 1.99$^{b}$ | 4.54 ± 1.58$^{d}$ | 9.24 ± 2.16$^{a}$ | <0.001 | <0.001 | <0.001 |
| C* liver | 10.98 ± 2.12$^{ab}$ | 10.25 ± 3.06$^{b}$ | 10.83 ± 2.59$^{ab}$ | 12.41 ± 3.08$^{a}$ | 11.48 ± 1.84$^{ab}$ | 12.83 ± 2.71$^{a}$ | 0.06 | 0.006 | 0.034 |
| h*(º) liver | 22.75 ± 8.00$^{c}$ | 37.68 ± 9.70$^{b}$ | 18.36 ± 5.72$^{c}$ | 40.80 ± 14.25$^{b}$ | 22.89 ± 7.68$^{c}$ | 46.25 ± 4.10$^{a}$ | <0.001 | 0.004 | 0.021 |

Different superscripts $^{(a, b, c, d)}$ in the same row indicate significant differences between groups ($p < 0.05$). SEM refers to the standard error of the mean. 0D (no fasting), 50D (65.49 ± 0.22 °C d in summer for 3 days and 58.7 ± 2.21 °C d in winter for 6 days), 100D (131.34 ± 0.07 °C d in summer 6 days and 114.5 ± 1.86 °C d in winter 13 days). S: summer, W: winter, Se: season and F fasting.

observed between fasting groups in summer, suggesting acclimation to higher temperatures. However, chroma values were higher in summer than in winter, with 100D groups in both seasons reaching similar levels, possibly linked to stress responses. Additionally, 100D winter group showed lower lightness (L*), likely due to the dispersing effect of adrenocorticotropic hormone on chromatophores. Similarly, liver color in summer remained unchanged across fasting groups. L* and redness were not affected by fasting but were influenced by seasonality, whereas chroma and hue were higher in winter. These changes are likely due to metabolic responses, mobilizing hepatic reserves and altering lipid composition. Overall, when fasting is the primary stressor, an initial acute stress response is followed by gradual metabolic adaptation, reducing stress over time. However, in summer, prior acclimation to higher temperatures lessens the response to additional stressors

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# EVALUATION OF *ALLIUM*-DERIVED EXTRACTS AS FUNCTIONAL INGREDIENTS IN DIETS FOR EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

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**Aim of research**

Sustainable aquafeed development is a growing priority in European seabass (*Dicentrarchus labrax*) farming. The incorporation of functional ingredients with antimicrobial or immunomodulatory potential must be validated to ensure optimal growth, welfare, and health status of farmed fish. In this context, organosulfur compounds from *Allium* spp., such as propyl propane thiosulfonate (PTS) and propyl propane thiosulfinate oxide (PTSO), have shown promising bioactivity. This study aimed to evaluate the protective and functional effects of PTS/PTSO using a dual approach: first, by assessing their anti-infective potential in an in vivo surrogate model (*Caenorhabditis elegans* infected with *Vibrio harveyi* and *V. anguillarum*), and second, through a 12-week nutritional trial in seabass juveniles reared in a recirculating aquaculture system, including evaluation of innate immune parameters and survival following *V. anguillarum* challenge.

**Materials and Methods**

The effect of pathogen exposure on *C. elegans* survival with and without PTSO was evaluated. Synchronous L4-stage nematodes were fed with *E. coli* OP50 (control), *V. anguillarum* LMG/1086, or *V. harveyi* 16/00. Three replicates were used per dietary condition (10 nematodes each). Each diet was tested with or without PTSO at 27.5 $\mu$M. Survival was analyzed using Kaplan–Meier and log-rank tests.

**RNA Extraction and RNASequencing.** Total RNA was extracted using TRIzol (Invitrogen, USA). RNA sequencing was performed on the NOVOGENE platform (UK). Differentially expressed genes (DEGs) were annotated with Gene Ontology (GO).

**Feeding Trial in European Seabass.** A 12-week feeding trial was conducted with 300 seabass juveniles (initial body weight: 3.25 ± 0.33 g) randomly distributed across 18 tanks (100 L) in a RAS. Two diets were tested in triplicate: (1) control diet (standard commercial feed), and (2) diet with *Allium*-derived extract blend. All feeds were isoproteic, isolipidic and isoenergetic. Zootechnical parameters and immune endpoints were monitored. After feeding, innate immune parameters (protease, antiprotease, lysozyme activity, total proteins) were assessed using commercial kits based on biochemical and enzymatic reactions. Fish were then challenged with *V. anguillarum* ($1\times10^4$ CFU/mL, immersion), and mortality was recorded for 14 days. Survival was analyzed by log-rank test.

**Results**

**Effect of *Vibrio* sp on *C. elegans* Lifespan.** Log-Rank test (OASIS2) showed significant survival differences. *V. anguillarum*- and *V. harveyi*-fed nematodes had reduced survival vs. *E. coli* OP50 (Bonferroni-adjusted $p < 0.05$).

With PTSO, *C. elegans* survival improved across treatments. Lifespan in *E. coli* + PTSO (18.84 days) was similar to control (19.72 days), suggesting no toxicity. Survival increased significantly in nematodes fed with *V. anguillarum* and *V. harveyi* + PTSO (16.91 and 15.32 days) vs. without PTSO (9.37 and 5.96 days).

**RNAseq Analysis.** NMDS analysis showed that PTSO altered transcriptomic profiles, reducing divergence among worms exposed to *E. coli*, *V. anguillarum*, and *V. harveyi*.

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Transcriptomic data showed downregulation of genes linked to epithelial defense, stress response and immune effectors (C-type lectins), and upregulation of genes involved in intracellular signalling, lysosomal metabolism, energy regulation and detoxification (e.g., cytochrome P450s). These data support a role for PTSO in modulating pathogen-induced stress and enhancing adaptative responses.

**Seabass Trial.** Although no significant differences were found in growth or immune parameters, fish fed the *Allium*-supplemented diet showed significantly higher survival after *V. anguillarum* challenge ($p < 0.05$), indicating enhanced disease resistance.

**Conclusion**

This study demonstrates that organosulfur compounds derived from *Allium* spp. exert protective effects against bacterial infection in a *C. elegans–Vibrio* model. Furthermore, their inclusion in pre-growing diets for European seabass has proven to be safe, with no negative impact on zootechnical performance. Compounds such as PTS and PTSO represent a promising functional strategy for aquafeeds, as they help maintain growth performance in European seabass while enhancing resistance to pathogenic infections in surrogate models. These findings also support the protective effects of these compounds in seabass, as evidenced by improved survival after *V. anguillarum* infection.

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# EVALUATION OF THE *Scophthalmus maximus* MICROBIOME AND ITS ANTIVIRAL RESPONSE TO VIRAL HEMORRHAGIC SEPTICEMIA VIRUS AFTER TREATMENT WITH OXYTETRACYCLINE

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**Introduction**

In recent years, the study of commensal microbiota has gained increasing relevance, as endogenous microbial communities are now recognized as key modulators of disease progression, including viral infections. These microbiota-host interactions play a complex, bidirectional role in the establishment and outcome of infections. In particular, the gut microbiota and its metabolites have been shown to influence the host's immune response and antiviral defenses (Mizutani et al., 2022).

Turbot (*Scophthalmus maximus*), a commercially important flatfish species, holds significant economic value in Spain, one of the world's leading producers alongside China. The intestinal microbiota of turbot comprises a diverse community of microorganisms, including both non-pathogenic and potentially pathogenic *Vibrio* spp., which under specific conditions can cause vibriosis—a major bacterial disease in aquaculture responsible for considerable economic losses.

Oxytetracycline (OTC), a broad-spectrum antibiotic, is widely used in aquaculture to treat vibriosis. It remains one of the most commonly administered antibiotics for both prophylactic and therapeutic purposes and is often readily available to aquaculture operations (Sanches-Fernandes et al., 2022). However, the overuse of OTC has been linked to the emergence of drug-resistant *Vibrio* strains (Ji et al., 2020), raising concerns about the long-term sustainability of current disease management practices. In addition to fostering resistance, antibiotics like OTC may have unintended consequences on the host by disrupting the composition and function of the gut microbiota.

**Materials and methods**

In this study, we aimed to investigate the impact of OTC treatment—administered under routine aquaculture conditions via immersion (0.1 g/L for 1 hour over four consecutive days)—on the gut microbiota of turbot. Additionally, we assessed whether the resulting alterations in microbiota composition influence the host's immune response and survival following infection with viral hemorrhagic septicemia virus (VHSV). Viral infections were conducted either one day after the final OTC treatment or 30 days later, to determine whether the effects of the antibiotic were transient or sustained over time. The intestinal bacterial microbiota was also analyzed at the one-month time point to evaluate the recovery of the normal microbial community. Changes in gut bacterial composition were characterized using 16S rRNA amplicon sequencing. To further assess the systemic effects of OTC, transcriptomic profiles of intestine and head kidney samples from both uninfected and VHSV-infected fish (24 hours post-infection) were analyzed using RNA-Seq.

**Results**

The results revealed a significant reduction in *Vibrio* spp. abundance across all OTC-treated individuals, with no detection of drug-resistant strains. The ecological niche vacated by *Vibrio* spp. was subsequently occupied by bacteria from various other taxonomic classes. This antibiotic-induced disruption of the gut microbiota had a marked effect on turbot survival following VHSV challenge: fish infected immediately after OTC treatment exhibited significantly reduced survival compared to untreated controls. In contrast, when infection was performed 30 days post-treatment, survival rates were comparable between treated and untreated groups, which is supported by a recovery of the gut microbiota and its associated protective functions over time.

At the transcriptomic level, RNA-Seq analyses revealed notable alterations in the expression of key immune-related genes in OTC-treated fish, which most likely contributed to their reduced survival following VHSV infection. These findings underscore the critical importance of maintaining microbiota integrity in aquaculture species. Beyond promoting antimicrobial resistance, the misuse or overuse of antibiotics can impair host immune defenses and increase susceptibility to viral infections.

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# ADVANCING *EX SITU* CONSERVATION METHODS FOR THE CRITICALLY ENDANGERED *Pinna nobilis*

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## Introduction

*Pinna nobilis* Linnaeus, 1758, is a critically endangered bivalve mollusk endemic to the Mediterranean Sea. Since the mass mortality event (MME) detected in 2016, the species has experienced drastic declines. Today, the only known surviving populations are restricted to areas where environmental conditions exceed the survival limits of the main parasite responsible for this mortality: *Haplosporidium pinnae* (Catanese et al., 2018). On the Spanish coast, one of the two remaining populations exists in the Mar Menor coastal lagoon (Murcia, SE Spain). However, this population has also suffered a sharp decline due to repeated episodes of eutrophication (Cortés-Melendreras et al., 2022). *Ex situ* maintenance of the species plays a key role in its conservation. However, this task has proven more difficult than initially expected, with no records to date of long-term maintenance or clearly defined optimal conditions. The objective of this study was to improve understanding of the optimal conditions for the *ex situ* maintenance of *P. nobilis* from the Mar Menor, at the Aquarium of the University of Murcia, from 2019 to 2023.

## Methodology

A total of 43 adult *P. nobilis* specimens were collected from the Mar Menor and transferred to the University of Murcia's Aquarium facilities from 2019 to 2023. The *ex situ* system consisted of four large-volume tanks filled with artificial seawater. Closed-loop maintenance tanks were equipped with a comprehensive filtration system combining mechanical, chemical, and biological filters, as well as UV germicidal sterilization and integrated temperature control. The optimal volume was about 200–300 liters of water per specimen in the tank. Individuals were kept in a vertical position within perforated mesh baskets. Environmental parameters from their natural habitat were replicated, including photoperiod, salinity, temperature, pH, and saturated dissolved oxygen levels. Alkalinity, calcium (450 mg/L), magnesium (1370 mg/mL), iodine (0.07 mg/L) were carefully monitored. Nutrient levels were maintained below 20 mg/L for nitrates and 1.5 mg/L for phosphates; values above these thresholds triggered complete water replacement. Seasonal temperature fluctuations from the Mar Menor were reproduced without exceeding stress thresholds (<14°C or >28°C). Environmental parameters were recorded daily between May 2019 and May 2023. Feeding protocols included a mix of three microalgae species cultivated in the lab: *Tisochrysis lutea* ($6\times10^3$ cells/µL), *Tetraselmis chuii* ($2\times10^3$ cells/µL), and either *Chaetoceros gracilis* or *Phaeodactylum tricornutum* ($7\times10^3$ cells/µL), combined in a 3:3 ratio. Additionally, zooplankton (*Brachionus rotundiformis* and *Acartia tonsa* nauplii at 0.5 units/mL) were provided. Of the 43 individuals, survival monitoring was performed for 22. Average survival times were calculated and monthly survival rates were also estimated based on the expected stay duration.

## Results

The average maintenance conditions from 2019 to 2023 are shown in Table 1. The recorded salinity values reflect those of the Mar Menor, remaining below the species' maximum tolerance (45 psu), but above the optimal level for *H. pinnae* infection (39 psu). The survival rate remained above 80% during the first 15 months, followed by a significant decline in the subsequent months (Fig. 1). However, from months 19 to 33, the number of surviving specimens remained stable until month 35, when another drop in survival was observed (Fig. 1). The average survival time was 19.43 ± 2.84 months, with one exceptional individual, collected in 2019, surviving for over 48 months.

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**Table 1.** Mean and maximum/minimum values of each parameter in the four systems between 2019 and 2023. Conversion of density to salinity based on temperature.

| | Density ($kg/m^3$) | Salinity (psu) | Temperature (°C) | Alkalinity (meq/l) | pH |
|---|---|---|---|---|---|
| **Mean** | 1031.04 ± 1.38 | 42-43 | 19.17 ± 2.06 | 3.10 ± 0.27 | 8.12 ± 0.07 |
| **Max.** | 1034.00 | >44 | 22.90 | 4.00 | 8.33 |
| **Min.** | 1028.00 | 39 | 15.36 | 2.20 | 7.84 |

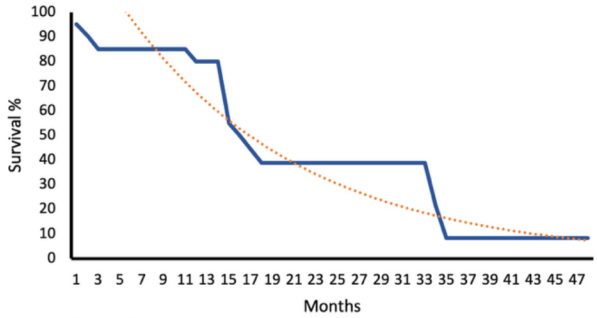


**Figure 1.** Monthly survival rate of maintained *P. nobilis* specimens.

## Discussion

The programmed and observed environmental parameters in the tanks are considered the most suitable for maintaining *P. nobilis* specimens from Mar Menor. Given the critical status of the population, minimizing stress on the individuals was a priority. Therefore, the natural environmental conditions in which the species completes its life cycle were carefully replicated in the *ex situ* system. Additionally, it is important to emphasize that environmental parameters should be tailored according to the origin of individuals. *Ex situ* conservation of *P. nobilis* has been marked by high mortality, primarily due to challenges in feeding and infections by pathogens like *H. pinnae* and *Vibrio mediterranei* (Prado et al., 2020). Infections by *Vibrio* spp. have been a major cause of mortality, especially under immunosuppressive conditions such as stress, wounding, or elevated temperatures. Nevertheless, in this study survival rates remain relatively high, and tank maintenance protocols for the species are gradually being optimized.

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# AUTOMATIC SIZE ESTIMATION OF SEA BREAM IN THEIR NATURAL UNDERWATER ENVIRONMENT USING DEEP LEARNING (DL)

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## 1. Introduction

This study leverages Deep Learning (DL) models and stereoscopic vision techniques to develop tools that automatically estimate fish size in near real-time, using a non-intrusive approach. Accurate and efficient length measurement in intensive aquaculture systems requires advanced object detection algorithms capable of extracting highly discriminative features specific to fish. However, practical fish detection still presents challenges, such as complex underwater backgrounds, varied fish orientations, and a lack of large-scale annotated datasets.

Monitoring fish growth is essential in aquaculture for optimizing feed quantity, oxygen supply, and overall animal welfare. Typically, length estimation relies on manual sample collection, a process that can induce stress, negatively affect fish growth and, in some cases, result in mortality. This highlights the urgent need for automated, non-invasive alternatives to manual handling. Stereoscopic vision techniques in computer vision have proven effective for estimating the size of freely swimming fish.

The primary objective of this work is to automatically process underwater stereo videos of freely swimming fish to obtain accurate measurements of many individuals in near real-time. The main contributions of the proposed approach are as follows:

- An encapsulated stereoscopic camera system was designed and prototyped to support long-term monitoring in underwater environments.
- A ground-truth dataset was developed, consisting of real images annotated with keypoints required for accurate analysis.
- A Convolutional Neural Network (CNN) was trained to automatically detect keypoints in the images, and this output was subsequently combined with stereoscopic vision to estimate fish length.
- Automatic measurements were validated through comparison with manual measurements.

## 2. Stereoscopic vision system and video acquisition

A stereoscopic camera system was prototyped using a watertight enclosure with sealed wiring, ensuring durability in aquatic environments. Its design required careful consideration of parameters such as focal length, inter-camera distance, shutter speed, and other optical and mechanical factors. Video recordings of sea bream at different growth stages were conducted at the Instituto de Acuicultura Torre de la Sal (IATS-CSIC) facilities, resulting in six hours of footage at varying resolutions and frame rates.

## 3. Results: Applying DL models to automatic fish length estimation

A dataset was generated by manually annotating the snout and tail keypoints of individual fish, resulting in 250 images and 700 annotated individuals. Two DL-based object detection models were trained: Keypoint R-CNN and YOLO. Keypoint R-CNN extends the Mask R-CNN architecture for keypoint detection. It first uses a Region Proposal Network (RPN) to generate candidate regions and then refines these proposals with a CNN-based object detection head. YOLO (You Only Look Once) is a fast, single-stage object detection algorithm that treats detection as a regression problem. It divides an image into a grid and simultaneously predicts bounding boxes and keypoints per cell in a single pass through the network, making it well-suited for real-time applications.

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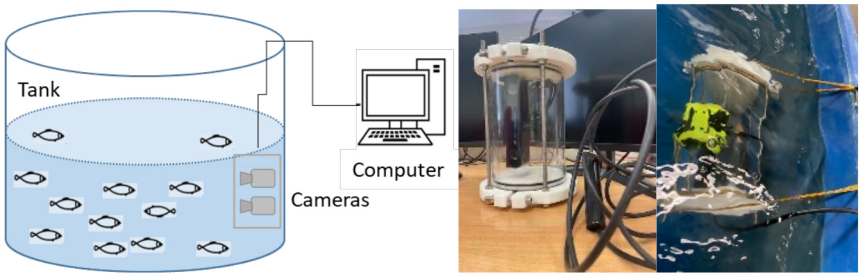


Figure 1. On-site data acquisition system.

| Tank Number | 25 | 27 | 15 | 25 |
|---|---|---|---|---|
| Date | 5/6/2023 | 27/9/2023 | 6/10/2023 | 6/10/2023 |
| Average Length (Manual) | 23.67 | 30.41 | 18.35 | 38.12 |
| Average Length (Keypoint-RCNN) | 23.91 | 30.00 | 18.15 | 36.18 |
| Average Length (YOLO) | 23.90 | 30.12 | 18.17 | 37.43 |

Table 1. Comparison of manual and automatic average fish lengths (in cm).

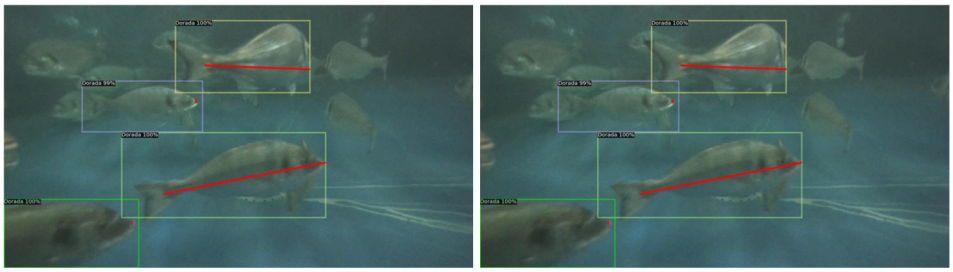

Figure 2. Automatic detection of sea bream lengths using Deep Learning and stereoscopic vision techniques.

Table 1 compares the average fish lengths obtained from manual measurements and the two DL models. Both models yielded accurate results, with YOLO performing slightly better, particularly in tank 25. The average processing times per frame were 0.347 ms for Keypoint R-CNN and 0.068 ms for YOLO. An example of automatic fish detection and length estimation is shown in Figure 2.

**4. Conclusion**

A dataset of sea bream was developed with manually annotated snout and tail keypoints to train Keypoint R-CNN and YOLO networks for automatic fish length estimation. Both models demonstrated high accuracy across different growth stages when compared to manual measurements. These results support the potential of Deep Learning and stereoscopic vision as a robust, non-invasive, and automated method for fish length estimation in aquaculture.

# ENVIRONMENTAL PERFORMANCE OF FARMED STURGEON CAVIAR PRODUCTION: AN LCA STUDY ON LUXURY FOOD

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## Introduction

Aquaculture is generally regarded as one of the most sustainable sources of animal-derived food (Gephart et al., 2021). Nevertheless, the variety of farmed species, technologies, and practices across regions requires product-tailored evaluations, especially for poorly studied luxury foods, such as caviar. Traditionally derived from wild female sturgeons, caviar is now primarily obtained through aquaculture due to the depletion of wild stocks (Bronzi et al., 2019). With 65 tons produced in 2023 (EUROSTAT, 2023), Italy ranks third in global caviar production, following China and Russia (EUMOFA, 2021). This study aims at (1) assessing for the first time the environmental performances of caviar production, including its co-products (i.e., meat), from different sturgeon species reared in a representative Italian farm, using the *Life Cycle Assessment* (LCA) methodology, and (2) evaluating, through scenario analysis, the effects of potential impact mitigation strategies.

## Materials and methods

The enterprise in northern Italy produces caviar from four sturgeon species: *H. huso*, *A. baerii*, *A. transmontanus*, and *A. gueldenstaedtii.* The production chain includes sturgeon farming and caviar/meat processing. Farming involves four sub-phases: nursery, on-growing, mixed males and females fattening, and females fattening. Sturgeons are raised in indoor recirculating aquaculture systems (RAS) and outdoor raceways. The water that feeds all the systems comes from a nearby freshwater channel. A sedimentation and biological filtration system is in place. The facility meets its energy needs using electricity from the grid and an in-house photovoltaic system (PV). As soon as males are identified (at 3-8 years, depending on the species), they are processed for meat, while females are raised until sexual maturity (8-20 years, depending on the species) for egg collection and meat processing. Eggs are processed, salted, and packed in tins or jars for sale.

The analysis used the Life Cycle Assessment (LCA) methodology (ISO 14040 and 14044), with a cradle-to-gate approach. The functional unit (FU) was defined as 1 kg of caviar (reference flow: 1 kg of caviar and 5.9 kg of marketable meat), with economic allocation applied. Primary data were gathered via questionnaires and site visits (reference production year: 2022–2023), while background data were sourced from Ecoinvent 3 and Agri-footprint 6.3. Calculations were performed using SimaPro 9.5, adopting ReCiPe 2016 Midpoint (H), Cumulative Energy Demand and CML-IA baseline methods. LCA's output allowed the identification of critical hotspots along the caviar production chain, which were also used to forecast two impact mitigation scenarios: 1) optimization of feed use by hypothesizing the application of a genetic early sex determination strategy (Kuhl et al., 2021), and 2) energy transition, i.e., by increasing the in-house PV power supply up to 50% of the energy needs.

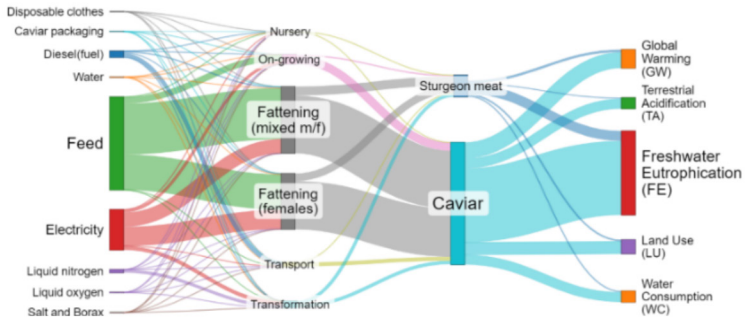


*Figure 1 Contribution analysis at the sub-phase, inputs and products levels for A. baerii*

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## Results

LCA results showed high variability depending on the species, e.g., Global Warming (GW) ranged between 53 and 83 kg $CO_2$ eq/kg of caviar, Terrestrial Acidification (TA) between 0.18 and 0.29 kg $SO_2$ eq/kg of caviar, Freshwater Eutrophication (FE) from 0.02 to 0.05 kg N eq./kg of caviar, Land Use (LU) from 43 to 93 $m^2$/kg caviar, and Cumulative Energy Demand (CED) from 714 to 1153 MJ/kg of caviar. For all the species, the fattening phases had the highest impact contribution, ranging from 80% in GW for *A. gueldenstaedtii* to 94% in FE and 99% in LU for *H. huso*. In all cases, the inputs that collectively accounted for >95% of the impacts were feed, electricity and transportation. Figure 1 depicts the contribution analysis at the sub-phase, inputs and products levels for *A. baerii*. The scenario analysis showed that: i) an early genetic sex determination allows a significant reduction in the feed used with consequent decrease in impacts (e.g., up to -21% of the impacts related to Freshwater Eutrophication-FE); ii) increasing the supply of renewable energy at the farm level is effective, but to a different extent depending on the species considered (from -2% to -19 % for GW).

## Discussion and Conclusions

Caviar production comes with significant environmental impacts, although mitigation strategies could improve its sustainability. Species-specific differences are mainly driven by farming cycle duration, feed requirements, and production efficiency. The fattening phase emerged as the primary contributor to impacts due to its length and resource intensity, with feed identified as a key hotspot, as also reported by Bohnes et al., 2019 and Gephart et al., 2021. Electricity was the second major hotspot: substituting grid electricity with solar energy reduced GHG emissions but increased abiotic resource depletion. Despite this burden shift, renewable energy remains essential for decarbonising the sector (De Vet et al., 2024). This study also underscores the methodological challenges in assessing complex systems through LCA, such as, for example, the choice of the appropriate FU, system boundaries, and temporal coverage for data collection.

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# LASTING EFFECTS OF FEEDING PLANT-BASED DIETS AFTER EARLY NUTRITIONAL INTERVENTIONS IN *Seriola dumerili*

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Introduction

Due to an increasing consciousness to provide more adequate production conditions, thereby ensuring animal welfare and high protein quality for the consumer, one of the biggest challenges is to assure the availability, sustainability and lowest costs of conventional feeds, mostly for carnivorous species as greater amberjack (*Seriola dumerili*). Despite recent advances in feed formulations, a significant gap remains in the ability to drive farmed fish development toward desired phenotypes. In particular, there is currently no protocol to prepare the intestinal physiology of fish from larval stages to the successful introduction of vegetable ingredients during the production cycle. However, some improvements related to developmental programming have been recently achieved. Thus, the use of several vegetable sources and their physiological implications in the early-life stages has been evaluated in the gilthead sea bream by using a high content of soybean meal as an epigenetic trigger (Perera and Yúfera, 2017), but the later consequences along the production cycle in juvenile/adult fish remain unknown. The present study evaluates the effects of dietary incorporation of a multibiotic nutraceutical extracted from algae during first-feeding of *S. dumerili*, and their lasting effects on growth and intermediary metabolism after being fed with a plant-based diet during the on-growing cycle.

Materials and methods

Fry specimens (~0.3 g of initial mean body mass) of *S. dumerilli* were obtained from commercial sources (Futuna Blue S.L., El Puerto de Santa María, Spain) and maintained at the indoor facilities of the *Instituto de Ciencias Marinas de Andalucía* (ICMAN-CSIC, Puerto Real, Spain) by feeding fish (90 days) as follow: i) a control group fed enriched artemia with *Tetraselmis chuii* (10 days) + a commercial formulations (CT), or ii) this same protocol with enriched artemia and aquafeed supplementation (1 %, BP) with the multibiotic extract from algae *Prebiodo II* (Biotechnology Biopolym S.A.). After that, fish were pit-tagged to be maintained in a common garden and fed with a commercial diet for 6 months, and then 1) nutritionally challenged with a plant-based diet (PP) or 2) fed with the same commercial diet (COM) for an extra 3-month feeding period. Growth and somatic indices were analysed along with plasma, muscle and hepatic metabolites (glucose, glycogen, lactate, triglycerides, cholesterol and proteins).

Results and discussion

Fish fed with *Prebiodo II* during first feeding (BP group) exhibited higher specific growth rates (SGR) 9 months after early-nutritional interventions, regardless of the experimental diet (COM or PP) fed during the on-growing cycle. BP-COM group showed the highest growth performance, followed by the same pattern by both CT-COM and BP-PP, being the lowest SGR registered for CT-PP fish. Regarding somatic indices, mesenteric fat (MSI) was very low (up to 0.35 %) and did not show significant differences among experimental groups, although those fish fed with BP diet (BP-COM and BP-PP) presented the lowest levels. Moreover, hepatosomatic index (HSI) and the intestine length index (ILI) revealed a significant increase in fish from the CT-PP group. This effect on ILI was accompanied by a constant epithelial resistance (Rt, W.cm$^2$) and higher apparent permeability in the anterior intestine of fish fed *Prebiodo II* during early nutritional interventions.

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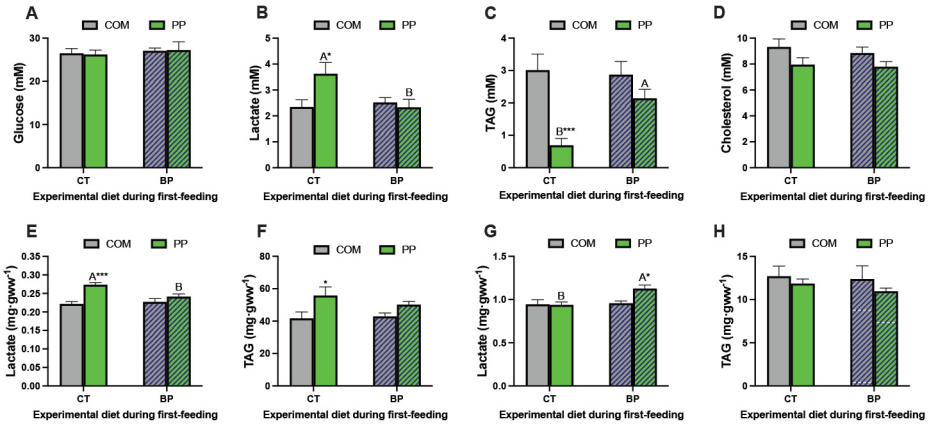


**Figure 1.** Plasma (A, B, C, D), hepatic (E, F), and muscular (G, H) metabolites of *S. dumerili* juveniles fed different experimental diets (COM and PP) after being fed during first-feeding without (CT) or with (BP) nutraceutics. Data are the mean ± SEM of 10 fish. Different superscript letters indicate significant differences among first-feeding dietary treatments (CT vs BP) for the same experimental diet (COM or PP). Asterisks represent differences between experimental diets (COM vs PP) for the same first-feeding dietary treatments (CT or BP) based on two-way ANOVA and Tukey's test (*p < 0.05; ***p < 0.001).

Attending to metabolic orchestration, plasma glucose maintains a homeostatic state along the four experimental sub-groups (Fig. 1A), whereas higher lactate (Fig. 1B) levels can also result from carbohydrate-enriched diets that contribute to increased glycogen stores throughout the formation of pyruvate from lactate by lactate dehydrogenase (LDH) at hepatic level (i.e. Cori cycle; Fig. 1E) and preferential glycogen degradation for energy requirements, as also shown herein. Our results also suggest that these observations can be found only in fish non-previously fed with *Prebiodo II*, where this compound appears to differentially regulate this metabolite in muscle (Fig. 1G). In addition, the contrasting trends for plasma TAG and cholesterol concerning lactate levels further indicate a shift in the energy substrate preferentially used, and thus an overall change in the relative contribution of anaerobic metabolism and TAG oxidation clearly ameliorated by early nutritional interventions (Fig. 1C and 1D). Moreover, our results suggest that plasma TAG depletion is the cause or the consequence of a preferential accumulation of this metabolite in the liver (Fig. 1F), but not in muscle (Fig. 1H) in fish fed PP-based diets and not supplemented with the nutraceutical compound during early stages.

## Conclusions

Our preliminary results demonstrate that carnivorous fish, genetically- and physiologically-adapted to high content of animal proteins in the diet, can be programmed by nutritional interventions, as that produced by the inclusion of a blend of different algae extracts biotechnologically obtained (*Prebiodo II*), and included in live crops and first microdiets during early development to better use vegetal proteins in later life during the on-growing cycle.

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# THERMAL AND CHEMICAL PRIMING FOR RESILIENCE: A MITIGATION STRATEGY AGAINST MARINE HEATWAVES

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University of Padova
Galilean School of Higher Education

The Manila clam (*Ruditapes philippinarum*) is a bivalve species with a broad native and introduced distribution holding significant economic and ecological importance. It is one of the most valuable species in global seafood markets and is widely used as a sentinel organism for environmental monitoring.

Climate change, characterized by a rise in average global temperatures, is one of the major global issues and has increasingly adverse effects on marine life. In particular, marine heatwaves — defined as prolonged periods of abnormally high seawater temperatures and closely linked to anthropogenic climate change — have been shown to impact marine life at multiple biological scales, including individual organisms, species, populations, and ecosystem services. These extreme events have been linked to alarming mass mortality events in bivalve populations. In this context, the development of effective mitigation strategies is urgently needed.

One promising approach is priming, described as the controlled exposure to a sublethal stress to enhance an organism's ability to cope with future environmental challenges.

This study focuses on testing different priming strategies on Manila clam. Both thermal and chemical sub-lethal stresses were used as priming agents.

Clams were divided into four groups that underwent the following treatments, with a duration of 7 days: no treatment (control), heat priming, chemical priming (hydrogen peroxide), and the combination of the two. After a period of resting (7 days), clams experienced a 7 days challenge during which they were exposed to daily 34°C heatwaves. The clams' digestive glands were sampled prior to priming, after priming, and after challenge.

Clam performance was evaluated using the following methods: survival monitoring over the following 14 days, hepatosomatic index (HSI) and condition index (CI), burrowing test, and transcriptional/biomarker analysis of samples.

To evaluate the effectiveness of priming as a mitigation strategy, the same treatment was applied to a second batch of clams for field testing. This approach aimed to assess the animals' responses to summer heatwaves under natural environmental conditions, thereby validating the potential of priming in realistic settings.

# EXPLORING GONADAL FACTORS IN EARLY GAMETOGENESIS AND PUBERTY: INSIGHTS FROM THE EUROPEAN SEA BASS

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## Introduction

Effective control of reproduction is fundamental for sustainable aquaculture of farmed species, and understanding in-depth the molecular regulatory systems governing gametogenesis becomes essential. In vertebrates, the brain-pituitary-gonad (BPG) axis regulates reproduction though gonadotropins, sex steroids, and local gonadal factors, the latter being largely unknown in fish. Among these, some members of the transforming growth factor β (TGF-β) family, including the Anti-Müllerian Hormone (Amh) and the Gonadal Soma-Derived Factor (Gsdf), are emerging as key regulators of early stages of gametogenesis and sex determination and differentiation [1]. To date, their characterization links them to male sex differentiation [2,3] and to the proliferation of type A spermatogonia (SgA) [4,5], while their regulatory roles in male and female adult gonads remains to be clarified. The European sea bass (*Dicentrarchus labrax*), an economically important species in Mediterranean aquaculture, provides a robust experimental model for this research based on the good number of tools available for reproductive physiology studies. Based on our previous knowledge and developed tools (sea bass recombinant proteins and specific antibodies) [6,7], the aim of this work is the functional characterization of Amh and Gsdf in male and female sea bass during puberty and adult gametogenesis.

## Materials and methods

To further investigate on these gonadal factors, a multidisciplinary approach including molecular, biochemical and physiological analysis was performed. Recombinant sea bass Amh and Gsdf produced in CHO cells were used to treat *in vitro* cultured testes and ovaries to assess their direct effects on the gonads. Specific antibodies allowed protein detection in gonadal extracts and their localization in histological sections to disclose the role of these proteins in the local control of gametogenesis. *In vivo* administration of hormone-coding plasmids were conducted in the pre-meiotic stage animals to analyse their action at the organism level. Finally, transactivation assays were done in Cos7 cells for studying Amh and Gsdf intracellular signalling pathways, and to identify the specific receptor for Gsdf, which remains unknown.

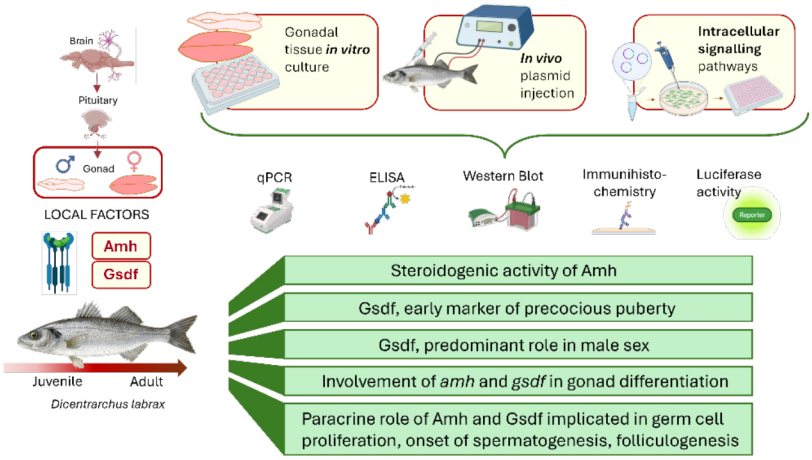


**Figure 1.** Graphical summary of the main outcomes of this study. Created in https://BioRender.com.

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**Results**

*Gene expression:* During juvenile gonadal differentiation, expression levels of *amh*, *amhr2*, *gsdf1* and *gsdf2* peaked at 250 days post-hatch. In testis of 1-year-old males that enter precocious puberty, the expression of *gsdf1* and *gsdf2* decreased compared with their siblings that remained immature. In adult males, *gsdf1*, and to a lesser extent *gsdf2*, showed maximum expression levels in premeiotic testis, while in adult females, the expression levels of *gsdf* genes were lower than in testis, and *gsdf2* mRNA predominated in post-ovulatory ovaries and in isolated follicular cells.

*Protein localization*: In adult testis, Amh was immunodetected in Sertoli cells surrounding early germ cell generations, whereas Gsdf was localized in Sertoli cells surrounding SgA but exclusively in the central region of each lobule. In ovaries, Amh was present in follicular cells of both early and late-vitellogenic stages, while Gsdf presence was confined to pre-vitellogenic follicular cells. In addition, Amhr2 was detected in SgA of post-spawning and pre-meiotic testis and in follicular cells of pre-/vitellogenic ovaries.

*Functional analysis*: *In vitro* cultured pre-meiotic testis or pre-/early-vitellogenic ovaries treated with recombinant Amh revealed its steroidogenic activity, resulting in an increase of steroid production. In the same vein, *in vivo* intramuscular injection of Amh expression plasmid caused an increase of circulating sexual steroids. Transactivation experiments in Cos7 cells confirmed that, in addition to the canonical Smad pathway, the sea bass Amh/Amhr2 pair was able to signal through the main steroidogenic cAMP pathway and its action was inhibited by the cAMP antagonist Rp-cAMPS. Finally, all the sea bass TGF-β type I receptor genes were cloned and functionally tested in Cos7 cells in combination with type II receptors to define the precise intracellular mechanism of action of Amh and to find the specific Gsdf receptor.

**Discussion and Conclusion**

The novel steroidogenic activity of Amh found in our experiments in sea bass indicates that fish Amh may act differently from mammalian Amh, which inhibit Fsh-induced on steroidogenesis. Gene expression analysis indicates that Gsdf1/2 and Amh have a clear function in male gonad differentiation. Moreover, the gonadal expression of *gsdf* genes during the adult reproductive cycle suggests a predominant role in adult males rather than females. The *gsdf1* downregulation in precociously maturing males suggests its potential as an early marker and target for puberty regulation. Protein detection and cellular localization demonstrated a paracrine role of both factors in germ cell proliferation, in the onset of spermatogenesis before meiosis, in folliculogenesis, and during vitellogenesis. Finally, the description of the specific interactions between type II and type I receptors, and their particular signaling trough phosphorylation of Smad 2/3 or Smad 1/5/8 proteins contributes valuable insights into the TGFβ signalling network in teleost gonadal function.

**Funding**

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# ITALIAN POLICY PERSPECTIVES ON CURRENT CHALLENGES IN FISHERIES AND AQUACULTURE

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## Introduction and background

The growing demand for seafood and the pressing need to reduce the ecological impact of blue sectors have elevated the sustainability of fishing and aquaculture industries to the forefront of European and international policymaking agendas in the last decades (FAO, 2024). This shift reflects a broader commitment to balancing economic growth with environmental sustainability, driving comprehensive policy initiatives across the blue economy.

In line with the United Nations 2030 Agenda, European institutions have aimed to ensure the blue sectors' sustainable development by promoting innovative practices and improving the management of aquatic resources, a transformative process known as the "blue transition". In this context, the European Maritime, Fisheries and Aquaculture Fund (EMFAF) aims to promote innovative projects and more effective governance strategies, backed by a financial allocation of €6.108 billion for the 2021-2027 period to support four key priorities: (i) sustainability of the fisheries sector, (ii) sustainability of aquaculture, (iii) development of maritime communities, and (iv) ocean surveillance.

The effectiveness of this instrument largely depends on its ability to address the actual needs of the sector's stakeholders (Vecchio et al., 2024) and bridge existing gaps between people and policy (Krause et al., 2015; Brugere et al., 2021).

In line with the abovementioned information, this study aims to analyze the blue sector stakeholders' perspectives on EMFAF 21-27's priorities in Italy to assess their views and alignment with current policies.

## Methodology

As an effective way to detect the viewpoints, the Q-methodology approach was applied, a quali-quantitative analysis aimed to identify the different perspectives within a group of participants, also known as discourses (Brown, 1980).

Following the steps of methodology (see McKeown and Thomas, 2013), 36 statements (Concourse) related to each priority of Italy's National EMFAF Plan (European Commission, 2021) have been designed. Afterwards, 18 respondents (P-sample) are called to rank the statements based on a predefined scale ranging from -5 to +5 (Q-set) based on their level of agreement or disagreement, arranging them within a normally distributed grid (Q-sorting) (Watts and Stenner, 2012).

The chosen participants are stakeholders who cover different roles in the supply chain - producers, cooperatives, public institutions, trade associations, academics, and consultants - to capture the different views on the relevance of different strategies proposed within EMFAF priorities. Lastly, a principal component analysis was performed to identify shared opinion patterns.

## Results

The Q-methodology analysis reveals five distinct perspectives, each emphasizing different opinions towards their alignment with current policies.

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The first discourse, "Cooperation and control", emphasizes the importance of regulation and marine area monitoring.

The second discourse, "Innovation, entrepreneurship, and a circular approach to aquaculture", demonstrates a strong interest in adopting innovative technologies and fostering new entrepreneurial opportunities in blue-farmed sectors.

The third discourse, or "Systemic vision and openness of mind", underlines the importance of an integrated approach involving all supply chain actors and promoting sustainable and collaborative marine resource management.

The fourth discourse is named "Local promotion and collaboration" since it highlights the significance of the statements falling into the second and third priority.

The last viewpoint, called "Sustainable fishing development", prioritizes the development of the fishing sector through fleet modernization, port infrastructure improvements, workforce training, and adopting more sustainable fishing practices

From a policy perspective, the present study reveals a significant divergence between stakeholders' perceived priorities, highlighting the potential need to revisit the allocation of financial resources for the blue transition of Italy's fisheries and aquaculture sectors.

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# EVALUATION OF THE POTENTIAL FOR ANTI-PREDATOR STOCKING TO REDUCE CROP LOSSES DUE TO FISH PREDATION IN GREENSHELL™ MUSSEL (*Perna canaliculus*) FARMS

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Enormous losses of juvenile mussels are commonplace in mussel aquaculture worldwide. Fish predation is one important contributor to these losses in many mussel growing regions. The Greenshell™ mussel industry in New Zealand is particularly susceptible to fish predation, where farmers have reported losses of seed mussels of up to 100%. The current practice for seeding juvenile Greenshell™ mussels onto coastal farms is to deploy the mussels alongside a continuous longline growing rope enclosed in a cotton stocking which holds the mussels against the rope. The mussels subsequently attach to the rope with byssus threads before the cotton degrades. During this vulnerable period, the stocking may also help to protect the juvenile mussels from fish predators whilst they are unattached to any substrate. This study investigated whether differences in the strength and weave of three types of stocking (i.e., a 54-loop weave commonly used in New Zealand, a smaller and more tightly woven stocking used in shellfish hatcheries, and a 42-loop stocking that combines two weaves of cotton together) might affect the amount of fish predation on juvenile mussels that were newly seeded onto growing rope. In an experiment, the three types of cotton stocking were each subjected to three levels of predation by restricting fish access to the growing ropes seeded with juvenile mussels by attaching full, partial and no plastic mesh cages around the ropes. The number of mussels lost from each of the treatment combinations were assessed after 10, 31, and 42 days. At 42 days on average only 15.1% (± 1.7 SE) of the seeded juvenile mussels remained on those growing ropes subjected to full fish predation, with no differences in mussel losses among three types of socking (Figure 1). In contrast, on average 90.7% (± 2.1 SE) of the seeded juvenile mussels remained on growing ropes protected from fish predation by the full mesh cages, and 87.5% (± 3.7 SE) for partial cages. For both types of protective cages there was no difference in the numbers of remaining juvenile mussels among the three types of stocking. Remote underwater video camera observations confirmed Australasian snapper (*Chrysophrys auratus*) were feeding on the juvenile mussels from the dropper ropes without protective cages. These results show that physical protection by cages protects juvenile mussels from fish predation, while cotton stocking, regardless of the type used, is not effective for preventing fish predation. The results also show that the majority of mussel losses in the uncaged treatment occurred in the first 10 days of the experiment, indicating that vulnerability to predation may be associated with the initial lack of byssus attachment to the growing rope. These results point to a need to develop more effective methods for mitigating the high losses of seed mussels after they are seeded onto growing ropes.

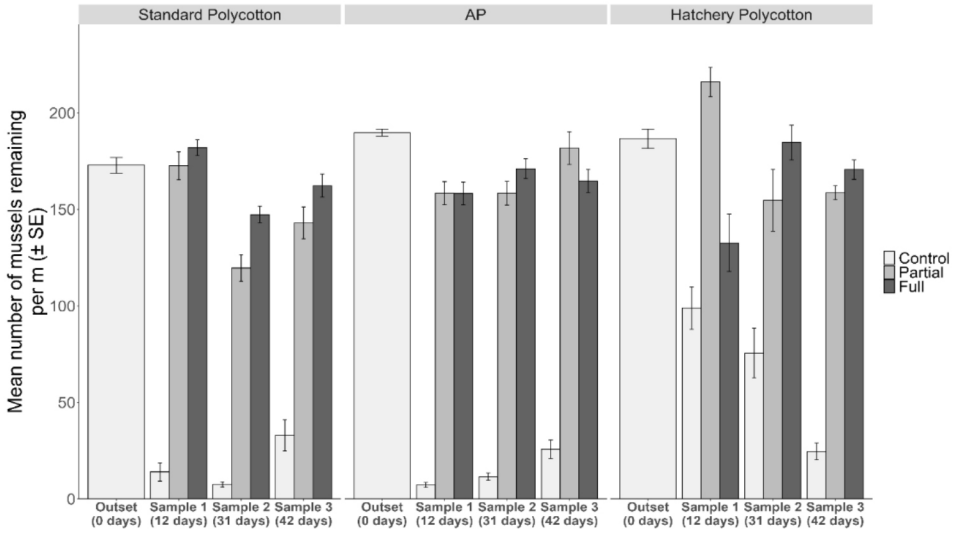


Fig 1. Mean number of mussels ($m^{-1}$ ± SE) remaining attached to dropper ropes in each of three stocking types and three cage treatments over the course of the 42-day experiment.

# FATTY ACID QUALITY IN FARMED FISH: EXPLORING THE USE OF INSECT MEAL

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## Introduction

In general, insect meal is increasingly recognised as an emerging, promising and sustainable raw material for fish and animal feed. Being the subject of extensive studies, current research lines focus on ensuring optimal growth performance and fish production with the aim to deliver fish with exceptional nutritional value. Fish is an important source of protein and fat for the human nutrition. The higher polyunsaturated fatty acid content (PUFA) of fish compared to other animal products has been associayed with the prevention of many chronic illnesses. In this context, this study evaluated the effect of dietary insect meal on the quality of the fatty acids in farmed sea bass and sea bream.

## Materials and methods

A detailed description of the feed composition and experimental conditions is given by Mastoraki et al. (2020, 2022). Briefly, four diets were designed and formulated for sea bass and sea bream. The control diet contained fish meal as the main source of protein (65% inclusion), while in the three experimental diets 30% of the fish meal was replaced by insect meal derived from *Tenebrio molitor* (TM), *Hermetia illucens* (HI, defatted) or *Musca domestica* (MD) larvae (19.5% inclusion). The diets were formulated to be isoproteic and isoenergetic, and for this purpose, the remaining ingredients were added in different amounts to each diet (fish oil was included in higher amounts mainly in the control diet and in the diet containing defatted HI). Sea bass and sea bream were reared in open flow indoor cylindrical tanks (500 L) for three months. At the end of the feeding trial, the whole body composition was determined and the indices were calculated from the fatty acid profile of the bodies (Mastoraki et al. 2020, 2022) as follows: Atherogenicity index (AI) = (aC12:0 + bC14:0 + cC16:0)/ (dP + eM + fM'); Thrombogenicity index (TI) = (C14:0+ C16:0+ C18:0)/ [(nM + oM' + p(ω-6) + q(ω-3) + (ω-3/ω-6)], where P is the sum of ω-3 and ω-6 fatty acids , M is the percentage of C18:1, M' the sum of the remaining monounsaturated fatty acids (MUFA), and a, b, c, d, e, f, n, o, p, q empirical constants (Ulbricht and Southgate, 1991); hypocholesterolemic / Hypercholesterolemic fatty acid ratio (h/H) = (C18:1 ω-9 + C18:2 + C18:3 + C20:5 + C22:5 + C22:6)/ (C14:0 + C16:0) (Santos-Silva et al., 2002); and the ratios characterizing the fatty acid profile PUFA/saturated fatty acids (SFA) and ω-6/ω-3.

## Results and Discussion

The fatty acid quality indices of the experimental fish are shown in Figure 1. The different diets, especially the different inclusion of fish oil in the diets, affected the ω-3 content of the fish. Thus, sea bass and sea bream consuming the FM and HI diets showed significantly lower ω-6/ω-3 ratios compared to the TM and MD groups. Substitution of FM with MD and TM led to a decrease in AI in sea bass due to higher MUFA and ω-6 fatty acid content. Partial FM substitution did not affect TI in sea bass as the index gives more weight to ω-3 fatty acids, which are present in higher levels in FM and HI fish. However, TI increased in sea bream fed TM compared to FM and HI. The higher the h/H index, the more beneficial the food is for protecting against cardiovascular disease. In this study, the highest h/H were observed in sea bass fed TM and MD, likely due to their higher content of C18:1 and C18:2 fatty acids (Mastoraki et al. 2020). In sea bream, the h/H index was not significantly affected by the different diets, possibly because the higher MUFA and ω-6 fatty acids levels in TM and MD fish were balanced with the higher ω-3 fatty acids in FM and HI fish (Mastoraki et al. 2022). In the present study, the sea bass fed with TM had significantly higher PUFA/SFA ratio compared to the other groups. The sea bream had a similar PUFA/SFA ratio among them. Fatty acid quality ratios in the fish body were within the appropriate limits for a healthy human diet, however, fish consuming non-defatted insect meal had lower ω-3 content.

## Conclusion

Fish fed with insect meals maintain suitable fatty acid profiles for human consumption. Defatting insect meal may enhance the nutritional quality of both the feed and fish by increasing the ω-3 levels. Insect meal in aquafeeds can indirectly affect the cardiovascular health markers depending on the fish species and the fatty acid composition of the fish diet. These findings highlight the importance of carefully selecting raw materials to preserve the nutritional value of the farmed fish.

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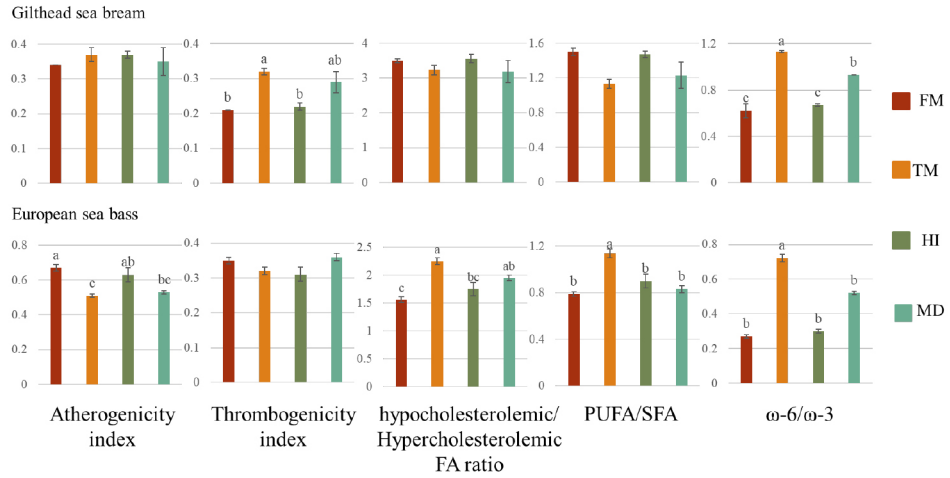
Figure 1. Fatty acid quality indices (mean ± standard deviation) of fish fed diets containing 19.5% of insect meals. Different letters in each graph indicate statistically significant difference among the diets (one way ANOVA, Tukey HSD, $P$<0,05).

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# IMMUNOMODULATORY EFFECT AND PERFORMANCE OF A DIETARY MIXTURE OF ARTEPILLIN C AND CURCUMINOIDS IN GILTHEAD SEA BREAM (*S. aurata*) UNDER COLD STRESS

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**Introduction**

Winter syndrome is a recurring condition affecting gilthead sea bream, causing significant mortalities across several Mediterranean regions during specific cold periods (<13°C) over the years. Is characterized by reduced feed intake and impaired growth while the causes of mortality may include not only thermal stress but also metabolic dysfunction, immunosuppression and opportunistic pathogens (Ibarz, et al. 2010). One potential strategy to reduce the adverse effects of the winter conditions is the inclusion of dietary functional ingredients intended to enhance fish immune status. This study evaluates the effects of Phyto AquaNity, a natural additive blend of Artepillin C (Green propolis) and curcuminoids (turmeric) on growth performance and immune support under water temperature variation condition.

**Materials and methods**

Fish (71.64g ± 0.58) were fed a control diet designed to resemble a commercial formulation or diets containing 0.1% Phyto AquaNity at optimal temperature (23°C) for 55 days. Then the water temperature was decreased by 1°C per day until it reached 13°C, where it stayed for 30 days. The trial was performed in triplicate within a recirculating aquaculture system (RAS). A transcriptomic and qPCR analysis of several immune response genes were performed at distal intestine samples from fish taken at the end of each rearing phase.

**Results and Discussion**

At 23°C, daily weight gain (DWG) and specific growth rate (SGR) were numerically higher in the treatment group compared to the control group, although the differences were not statistically significant ($p>0.05$) and the feed conversion ratio was similar between the groups (Table 1). At 13°C, fish fed Phyto AquaNity showed significantly higher SGR and DWG compared to the control group ($p<0.05$).

Transcriptomic analysis revealed that Phyto AquaNity influenced the expression of more than 290 genes at 13°C. The combination of Artepillin C and curcuminoids did not show any notable gene regulation changes compared to the control group in the higher temperature. However, at cold temperature a significant body of genes were regulated involving the enrichment of immune and metabolic pathways including the enrichment of the beta-alanine metabolism pathway.

The results of the qPCR analysis of the gene expression of immune response genes are presented in Figure 1. At 13°C, key inflammation associated genes (B2M, Cats and NCCRP) were down-regulated when fish ate Phyto AquaNity. Specifically, HSP70 and NCCRP were significantly down-regulated at 13°C.

The results suggest that dietary Phyto AquaNity may enhance the immune and metabolic pathways, while helping to mitigate inflammation and cellular stress responses in fish, potentially enhancing growth performance during rearing at low temperatures.

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**Table 1**: Growth and feed utilization efficiency of Gilthead Sea bream fed with Phyto AquaNity.

| | 23°C | | 13°C | |
|---|---|---|---|---|
| | **Control** | **Phyto AquaNity** | **Control** | **Phyto AquaNity** |
| **IW** | 71.01±0.34 | 71.02±0.46 | 174.43±0.67 | 177.64±2.31 |
| **FW** | 172.5±0.76 | 179.23±1.32 | 203.8±1.75 | 212.7±0.96 |
| **DWG** | 1.83±0.02 | 1.97±0.02 | 0.74±0.04[a] | 0.88±0.04[b] |
| **SGR** | 1.62±0.01 | 1.68±0.01 | 0.39±0.02[a] | 0.45±0.03[b] |
| **FCR** | 1.11±0.02 | 1.13±0.01 | 1.32±0.04 | 1.33±0.04 |

IW: Initial weight (g), FW: Final weight (g), DWG: Daily weight Gain (g), SGR: Specific Growth rate (%/day) and FCR: Feed Conversion Ratio. Different letters for each temperature in the same row indicates significant difference between experimental diets ($p<0.05$). Average ± standard deviation (n=3)

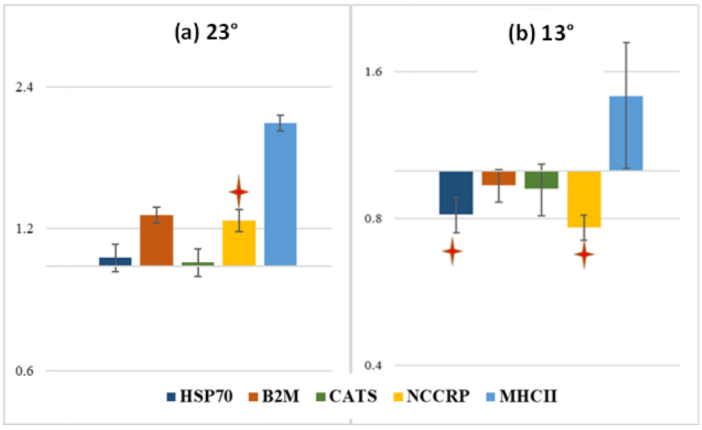


Figure 1. qPCR expression results of immune response genes of the distal intestine of fish fed Phyto AquaNity. Red star indicates significant when compared to the control feed. x-axes in log2 scale.

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# MUSSEL AQUACULTURE *(Mytilus galloprovincialis)* IN THE EBRO DELTA (NW MEDITERRANEAN)

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## Introduction

In the context of climate change, the warming of the Mediterranean Sea, along with the increased frequency of heat waves, poses a high significant risk to mussel farming. High water temperatures (above 28ºC) lower the physiological performance and may result in mass mortality events. Mussels (*Mytilus galloprovincialis*) cultured in enclosed environments are especially vulnerable to climate change, compromising ecosystem goods and services such as good water quality, food provision and the socioeconomics in the area.

## Material and Methods

To investigate the effects of temperature in the performance of *M. galloprovincialis,* field experiments were carried out monthly from March to July 2025 in the mussel farms from Fangar Bay (Ebro Delta, NW Mediterranean Sea) following the expected increase of temperatures from spring to summer. Feeding behavior of cultivated mussels was determined using the biodeposition method as described in Galimany et al., 2011, estimating parameters such as clearance, ingestion and absorption rates. Temperature was recorded continuously and water quality, according to the particulate matter available for the mussels, was evaluated monthly during the feeding experiments. Other biological parameters such as valvometry, biomarkers and the histopathological condition of the mussels were also evaluated although the results are not reported in this abstract. Linear models and Generalized Additive Models (GAMs) were used to model the feeding behavior response to temperature.

## Results and Discussion

The results showed that the water temperature increased from 14.4ºC in March to 27.6 ºC in July. In response to this increase in temperature, mussels exhibited a tendency to reduce both their clearance and absorption rates. The clearance rate exhibited a pronounced decline as temperature increased, followed by a stabilization around 20ºC, suggesting a non-linear physiological response to thermal stress (Fig. 1A). Nonetheless, the absorption rate which is dependent on the organic content of the water, decreased linearly with rising temperatures, indicating a consistent negative physiological response when water quality is also considered (Fig. 1B).

The results obtained during the experiments are similar to others found for the same specie (Anestis et al., 2010) and specific area (Galimany et al., 2011) where physiological performance decrease significantly when temperatures exceed 20ºC. These findings highlight the sensitivity of mussels' behavior to thermal stress, emphasizing the potential impact of rising temperatures on the species' performance, survival and aquaculture production.

## Acknowledgement

This research has been conducted within the framework of the MoMeNTo project, undertaken in collaboration with the Fundación Biodiversidad, from the Ministry for Ecological Transition and the Demographic Challenge, through the Pleamar Program, and is co-financed by the European Union through the EMFAF (European Maritime, Fisheries and Aquaculture Fund).

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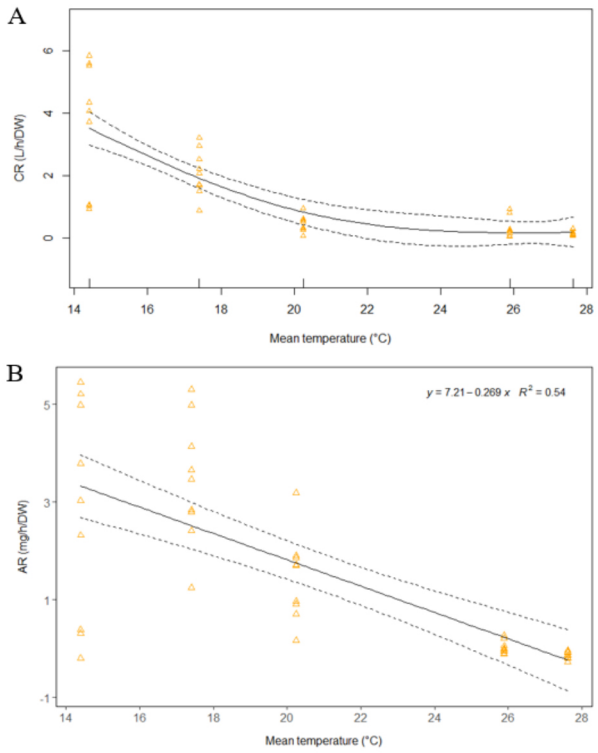


Figure 1. Clearance rate (CR) related to the temperature (A). Absorption rate (AR) related to the temperature (B).

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# SUPERIOR GROWTH PERFORMANCE IN CARP FRY ACHIEVED WITH CHITOSAN-ALGINATE ENCAPSULATED A-GHRELIN VERSUS FREE PEPTIDE

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**Introduction**
Ghrelin is a potent stimulator of growth hormone (GH) secretion and plays a key role in appetite regulation, metabolism, and immune modulation in fish. However, its oral delivery is limited due to degradation in the gastrointestinal tract. This study aimed to evaluate the efficacy of encapsulated A-Ghrelin using chitosan-alginate (Cs-Al) nanocarriers to improve its bioavailability and enhance growth, immunity, and stress response in common carp fry.

**Materials and Methods**
A-Ghrelin was synthesized via Fmoc-based solid-phase peptide synthesis and encapsulated into Cs-Al nanoparticles using ionic gelation. The particles were characterized by DLS (size, zeta potential), FTIR, FE-SEM, and AFM. In vitro release studies simulated gastric and intestinal pH. A 4-week feeding trial included six groups: control, free A-Ghrelin, A-Ghrelin-Cs, A-Ghrelin-Cs-Al, Cs-Al + A-Ghrelin, and Cs-Al alone. Growth metrics (weight gain, FCR, SGR), biochemical indices (cortisol, glucose, ALT, AST), immune parameters (IgM, C3, lysozyme), and gene expression (igf1 via qRT-PCR) were assessed. Histological analysis of intestines and gills was performed.

**Results**
Cs-Al nanoparticles (150 nm, +22 mV) showed 73.8% encapsulation efficiency and pH-dependent release (70% in 6 h). A-Ghrelin-Cs-Al significantly increased weight gain (2.3 g), SGR, and intestinal wall thickness (0.518 mm). GH and igf1 expression were elevated across all ghrelin treatments. Immune modulation was noted in Cs-Al groups, with reduced IgM and C3 in encapsulated ghrelin-fed fish. ALT and AST levels varied with treatments, indicating altered hepatic activity. 100% survival was recorded across all treatments.

**Conclusion**
Chitosan-alginate encapsulation effectively delivered A-Ghrelin, enhancing growth performance and physiological responses in carp fry. This nanocarrier approach shows strong potential for sustainable aquaculture through improved feed efficiency and peptide stability.

# DEGRADATION OF AQUATIC ECOSYSTEMS IN UKRAINE: WARTIME CHALLENGES

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**Introduction**

The ongoing war in Ukraine has precipitated significant environmental challenges, notably impacting aquatic ecosystems and posing substantial obstacles to sustainable aquaculture development. This study presents findings from a comprehensive assessment of water bodies across multiple Ukrainian regions, including Kyiv, Kherson, Mykolaiv, Chernihiv, Kharkiv, and Odessa.

**Materials end methods**

Hydrophysico-chemical analyses were conducted in Ukraine in accordance with the national standard of Ukraine "Water. Fishery water bodies. Ecological indicators of water quality" (SOU 73.1-41-050:2006), which establishes the requirements for ecological characteristics of water bodies suitable for fish farming. ons were conducted in accordance with the accepted classification of water quality in Ukraine The heterotrophic microorganisms (saprophytes) in the water body were found to be less than 0.5 thousand cells/mL, indicating a Class I (xeno-saprobic zone) status (very clean). The number of heterotrophs in the water body was found to be between 0.5 and 5.0 thousand cells/mL, indicating a Class II (oligo-saprobic) status (clean). The number of heterotrophs in the water body was found to be between 5.1 and 10.0 thousand cells/mL, indicating a Class III (beta-mesosaprobic) status (moderately polluted). The number of heterotrophs in the water body was found to be between 10.1 and 50.0 thousand cells/mL, indicating a Class IV (alpha-mesosaprobic) status (polluted); between 50.1 and 100.0 thousand cells/mL, indicating a Class V (beta-polysaprobic) status (very polluted) and more than 100.0 thousand cells/mL, indicating a Class VI (alpha-polysaprobic) status (extremely polluted. Structural indicators of ichthyofauna were studied using catch data from 2024. The material was collected from fixed gillnets and drift gillnets with different mesh sizes. Biological analysis was carried out using standard ichthyological methods.

**Results**

Hydrophysico-chemical analyses revealed elevated levels of organic pollutants in certain areas. For instance, the River Kozka in the Kyiv region exhibited biological oxygen demand (BOD) and chemical oxygen demand (COD) values exceeding permissible limits, indicating significant organic pollution. Additionally, increased mineralization was observed in Lake Sasyk (Odessa region), with chloride, sulphate, and iron concentrations surpassing standard thresholds.

In 2024, the Kosheva river (Kherson region) exhibited the highest degree of pollution, with a heterotrophic count reaching 16,000 cells/ml, indicative of an alpha-mesosaprobic status. In Kyiv region, the Chorne and Kalynivka lakes exhibited an increase in pollution, reaching 9.55 thousand cells/mL, which suggests an imminent ecological instability. The water bodies in the Mykolaiv region exhibited a stable status with a bacterial count of up to 5,500 cells/mL. In contrast, the ecosystems in the Odesa and Chernihiv regions demonstrated fluctuations from "clean" water in spring to "moderately polluted" in summer, with a peak of bacteria in June-August. The results indicate an intensification of water pollution in regions most affected by anthropogenic impact and the consequences of military actions. Furthermore, elevated counts of saprophytic and opportunistic microorganisms, including *Salmonella spp*. and Pseudomonas spp., were detected, reflecting deteriorating water quality and potential risks to aquatic life. The increase in saprophytic bacteria demonstrates a disturbance of ecosystem balance, leading to the transition of waters to higher saprobity classes. This confirms the irreversibility of degradation processes in aquatic ecosystems under the impact of military actions.

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A survey of the fish populations revealed a significant decline in commercial catch rates observed in the tested water bodies. A decline in species diversity from 18-19 to 13 species was observed in the Desna river (Chernihiv region), accompanied by a notable reduction in the bream population. These findings suggest that the ecosystem is undergoing degradation. A shift in the ichthyofauna structure was observed in the Kyiv Reservoir (Kyiv region), with a notable increase in silver carp dominance. This was accompanied by a decline in catches of pike, perch, bream, and roach, which is likely associated with deteriorating habitat conditions. In the Yuzhny Bug River (Mykolaiv region), the commercial fish stock declined by 95%, while natural fish mortality increased to 0.34, indicating the destruction of ecosystem processes. The analysis indicates serious degradation processes in the ecosystems of water bodies, manifested in the loss of biodiversity, reduction in the number and biomass of dominant species, as well as a reduction in the commercial potential of fish stocks. This decline was accompanied by a complete loss of the ecological services that these waters had previously provided.

The study highlights the multifaceted impact of armed conflict on Ukraine's aquatic environments, emphasizing the urgent need for integrated management strategies to mitigate environmental degradation and support the resilience and sustainability of aquaculture practices in wartime conditions. This requires rapid and effective solutions that take into account political, economic, environmental and social aspects.

The research was conducted as part of the NATO Peace and Security Science (SPS) project "Developing a Strategy for the Assessment and Restoration of War-Affected Aquatic Ecosystems" No. SPS G6085..

# HEMPSEED BY-PRODUCT AS AN ALTERNATIVE PROTEIN SOURCE FOR ATLANTIC SALMON (*Salmo salar*)

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## Introduction

The rapid expansion of aquaculture must be pursued sustainably, with an emphasis on using responsibly sourced raw materials that align with the circular economy and zero-waste principles. As a result, there is an ongoing search for more environmentally friendly terrestrial plant-based alternatives to traditional feed ingredients like fishmeal, fish oil, and even current substitutes such as soy. One promising option is hempseed, derived from industrial hemp varieties containing low levels of cannabinoids (THC < 0.3%). Grown domestically in the UK, and in Europe, industrial hemp offers environmental advantages, including higher CO absorption compared to many other commercial crops (Visković et al., 2023). Additionally, hempseed is a secondary by-product, further minimizing its carbon footprint, as the primary purpose of hemp cultivation is to produce construction and energy materials from the plant's stalks.

## Materials and Methods

Building on previous findings that demonstrated the favourable digestibility of hempseed in Atlantic salmon diets, the present 13-week study aimed to assess fish performance, fillet quality for consumers, and the effects of a hempseed-based diet on gut health. The trial compared two experimental diets: a control diet containing 15% soy protein concentrate (SPC) and a test diet in which SPC was fully replaced with 15% hempseed meal (HM). At the end of the trial all fish were humanely euthanized, performance and welfare indicators were recorded, and samples collected for biochemical, gene expression and histological analysis.

## Results

No significant differences were observed in growth performance, feeding parameters, or welfare between Atlantic salmon fed a commercial diet and those fed the experimental hempseed-based diet. Similarly, there were no notable differences in any of the nutritional parameters assessed. These findings suggest that hempseed is a viable substitute for SPC in salmon diets, offering benefits for both fish health and human consumption. Further analyses—including histological examination, short-chain fatty acid profiling, and gene expression studies—are underway and are expected to provide additional insights into the effects of hempseed on gut health.

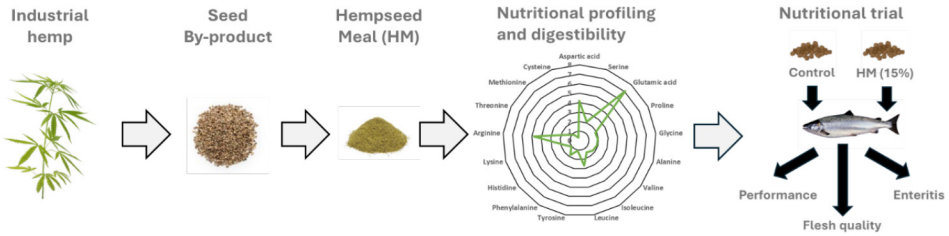


**Figure 1. Schematic of hempseed protein production and nutritional assessment.**

Visković et al., 2023; https://doi.org/10.3390/agronomy13030931

# ALTERNATIVE SUSTAINABLE AND ORGANIC PROTEIN SOURCES TO FISH MEAL IN WHITE SHRIMP *Litopenaeus vannamei* DIETS

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## Introduction

The shrimp aquaculture industry, particularly for *Litopenaeus vannamei*, relies heavily on fish meal as a primary protein source in feed. However, the sustainability and rising cost of fish meal have driven the search for alternative protein ingredients. Various animal- and plant-based proteins are being explored to reduce dependence on fish meal while maintaining growth performance and feed efficiency. Identifying effective, sustainable, and economically viable protein sources is crucial to support the continued expansion and environmental responsibility of shrimp farming.

## Material and methods

Six dietary formulations (isoproteic, 31% crude protein and isolipidic, 10% crud lipid) were formulated with organic ingredients and the fish meal was replaced by the following organic protein meals: organic chicken carcass (CAR), organic trout by-product (TRU), pig lung (PULCER), a chicken by-products mix (legs, heads and necks, PACACUE), a combination of all these ingredients (MIX), in addition to a control diet (CON) that included 13% fish meal. Growth trial was conducted over 99 days at the Aquaculture Laboratory of the Department of Animal Science, Universitat Politècnica de València.

The growth trial was performed in a recirculating aquaculture system (RAS) using 90 L tanks, with triplicate groups (n=3) per treatment. 16 juveniles *Litopenaeus vannamei* (initial mean weight 7.1 g) were distributed in 18 tanks. Shrimp were individually weighed and monitored every three weeks to assess growth performance, survival, and feed intake adjustments.

## Results and discussion

During the 99-day trial, all shrimp fed the experimental diets supported continuous growth (figure 1), increasing weight from 7.1 g to 16.4 g at the end of the study. The shrimp fed the trout by-product diet showed the highest final weight (17.7 g) and net gain (10.6 g), even so, all diets showed comparable effectiveness with the control diet.

Across all dietary treatments (table 1), no significant differences ($p > 0.05$) were observed in feed conversion ratio (FCR) and survival, indicating that full replacement of fishmeal with organic animal by-product proteins did not compromise feed efficiency or shrimp viability. Although without significant differences, the trout by-product diet (TRU) resulted in the highest final weight and the most efficient FCR ($1.96 \pm 0.28$).

Survival rates were generally high, with the CAR diet achieving 100% survival. Recent research further supports these outcomes. Fanttato et *al*. (2024) demonstrated that inclusion of insect meal and animal by-products maintains growth performance and enhances disease resistance. Together, these results, consistent with Candela-Maldonado et al. (2025), confirm the potential of alternative protein sources to replace fish meal sustainably without negatively impacting key production parameters in shrimp aquaculture.

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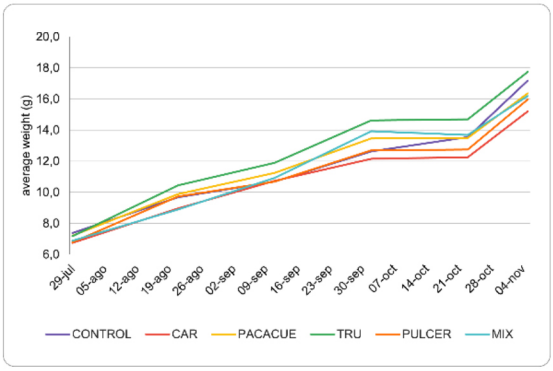


*figure 1. Evolution of the average weight of shrimp*

Table 1. Growth, nutritional parameters, and survival of shrimp at the end of the experiment (99 days)

| Diet | CONTROL | CAR | PACACUE | TRU | PULCER | MIX |
|---|---|---|---|---|---|---|
| **Initial Weight (g)** | 7.4 ± 0.3 | 6.8 ± 0.3 | 7.2 ± 0.3 | 7.2 ± 0.3 | 6.8 ± 0.3 | 6.9 ± 0.3 |
| **Final Weight (g)** | 16.4 ± 1.2 | 14.9 ± 1.1 | 16.5 ± 1.1 | 17.9 ± 1.1 | 15.7 ± 1.1 | 16.0 ± 1.1 |
| **Survival (%)** | 75.0 ± 7.9 | 100.0 ± 7.9 | 95.8 ± 7.9 | 89.6 ± 7.9 | 75.0 ± 7.9 | 83.3 ± 7.9 |
| **FCR** | 2.31 ± 0.28 | 2.35 ± 0.28 | 2.46 ± 0.28 | 1.96 ± 0.28 | 2.13 ± 0.28 | 2.31 ± 0.28 |

Values represent mean ± standard error (n = 3). For each parameter, values not sharing the same letter are significantly different ($p < 0.05$).
FCR: Feed Conversion Ratio = feed intake (g) / biomass gain (g)

## Conclusion

The results demonstrate that sustainable and organic animal protein sources can fully replace fish meal in *Litopenaeus vannamei* diets without compromising growth, feed conversion, or survival. The trout by-product diet exhibited the best performance, confirming its sustainable and economic viability. These findings support reducing fish meal dependency in shrimp aquaculture.

## Acknowledgements

This study forms part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (GVA-THINKINAZUL/2021/006; M. Jover; D. Sánchez).

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# IMPROVING NUTRITIONAL QUALITY OF SOYBEAN MEAL BY *Bacillus subtilis* SOLID-STATE FERMENTATION FOR SHRIMP FEED APLICATION

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## Introduction

Intensive aquaculture, particularly shrimp farming, necessitates effective and sustainable protein sources to use in the development of feed. Soybean meal is one of the most prevalent plant-based ingredients that is normally used as a fishmeal partial substitute, but it contains nutritional limitations for fish such as shrimp due to its low concentration of certain essential amino acids, the presence of antinutritional factors, and lower digestibility. Controlled fermentation of organic soybeans with *Bacillus subtilis*, was reported to improve their nutritional value by increasing the level of bioavailable proteins, improving the essential amino acid profile.

The current study evaluated the effect of organic soybean fermentation by B. subtilis on protein content, amino acid profile to enhance its application in shrimp feed.

## Material and methods

Two solid-state fermentation trials of soybean meal were conducted at the ICTA laboratory using a *Bacillus subtilis* bacterial inoculum. In the first experiment, an inoculum concentration of $5 \times 10^7$ CFU/mL was applied in volumes of 1, 5, 7.5, and 10 mL, maintaining a volume-to-weight (v/w) ratio of 1:1 (water : soybean meal). Fermentation was carried out at a constant temperature of 43 °C for 62 hours.

In the second experiment, a higher inoculum concentration ($1 \times 10^9$ CFU/mL) was used in volumes of 1 and 2.5 mL, with a water-to-substrate ratio of 1.5:1 (v/w). Fermentation was conducted over periods of 76 and 168 hours under controlled conditions. Then, a proximal nutritional composition analysis was performed to determine their crude protein level and aminoacid profile.

## Results and discussion

In both trials, *Bacillus subtilis* solid-state fermentation significantly increased the crude protein (CP) content of soybean meal (Table 1), from 50.37% in the pre-fermented control to 54.81–56.25% in the fermented treatments ($p < 0.05$). This improvement of 4.5–6.0 percentage points is consistent with the findings of Zhang et al. (2021), who reported an increase in CP from 50.72% to 55.71% following *Bacillus subtilis* fermentation, underscoring the influence of fermentation parameters on protein enrichment. In contrast, Yao et al. (2021) did not observe a direct increase in CP content; however, they demonstrated substantial improvements in protein quality through the marked reduction of anti-nutritional factors such as glycinin and β-conglycinin.

Extended fermentation (168 h) did not result in further increases in crude protein (CP) content, which aligns with findings from Zhu et al. (2023). In their solid-state fermentation study of soybean meal, Zhu and colleagues observed that prolonged microbial activity led to the degradation of macromolecular proteins with consequent heat generation and changes in nitrogen solubility and suggested that excessive fermentation time may provoke protein turnover and diminish CP gains.

Regarding the essential amino acid profile (Figure 1), the treatment involving fermentation of soybean meal with 1 mL of *Bacillus* inoculum over 62 hours (Trial 1) demonstrated notable increases in the concentrations of several key essential amino acids, including methionine, leucine, valine, phenylalanine, and isoleucine.

*(Continued on next page)*

Table 1: Crude protein expressed as dry matter percentage (CP (dm%)) of soybean meals before and after the fermentation process at a different condition.

| **1st trial with *bacillus* (5. 10^7 UFC/ml)** | **crude protein (dm%)** | **2nd trial with *bacillus* (1. 10^9 UFC/ml)** | **crude protein (dm%)** |
|---|---|---|---|
| prefermentend soybean meal | 50,37 ± 0,1 **a** | prefermentend soybean meal | 50,37 ± 0,1 **a** |
| Fermented Organic soybean -**A**- (1 ml of bcl; 62h) | 55,51 ± 0,36 **b** | Fermented organic soybean **FS 1** (1ml bcl; 76h) | 55,56 ± 0,55 **b** |
| Fermented Organic soybean -**B**- (5 ml of bcl; 62h) | 55,39 ± 0,11 **b** | Fermented organic soybean **FS 1*** (1ml bcl; 168h) | 54,81 ± 0,15 **b** |
| Fermented Organic soybean -**C**- (7.5 ml of bcl; 62h) | 56,25 ± 0,13 **b** | Fermented organic soybean **FS 2** (2.5ml bcl; 76h) | 54,92 ± 0,14 **b** |
| Fermented Organic soybean -**D**- (10 ml of bcl; 62h) | 55,91 ± 0,04 **b** | Fermented organic soybean **FS 2*** (2.5ml bcl; 168h) | 55,30 ± 0,61 **b** |

Values are presented as the mean ± standard error (n = 3). Values that do not share the same letter are significantly different ($p < 0.05$).

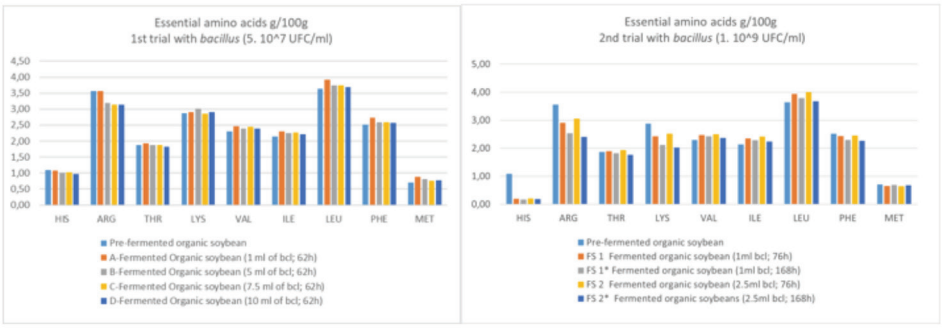


Figure 1: Essential amino acids of soybean meals before and after the fermentation process at different conditions.

## Conclusion

Bacillus subtilis solid-state fermentation effectively improves the crude protein content and essential amino acid profile of soybean meal, enhancing its nutritional value for sustainable shrimp feed formulation.

## Acknowledgements

This study forms part of the ThinkInAzul programme and was supported by MICIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (GVA-THINKINAZUL/2021/006; M. Jover; D. Sánchez).

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# NOVEL MICROBIAL PROTEIN SOURCE FROM *Methylococcus capsulatus* CAN REPLACE 50 % FISHMEAL OF A GILTHEAD SEA BREAM (*Sparus aurata*) DIET

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**Introduction**
Replacement of fishmeal in fish diets with the ongoing development of sustainable aquaculture is a global and lengthy task. A completely new industrial sector has arisen to produce valuable, novel, and alternative raw materials, among which different single-cell sources of protein could be highlighted. Coming from different fermentation technologies, these raw materials excel in terms of protein content and amino acid profiles, as well as possibilities to be functional ingredients (Hua et al., 2019; Mensah et al., 2025). The case of methanotrophic bacteria is one of the most promising. Because they use methane (natural gas) as a source of energy, companies such as Unibio A/S can produce protein while bypassing the typical cost fluctuations of agronomical practices.

The current study evaluated Uniprotein® (UP) as a fishmeal replacer in diets for gilthead sea bream (*Sparus aurata*). Growth and antioxidant performance were monitored to evaluate the potential of the ingredient for this fish species.

**Materials and methods**
A total of 800 gilthead sea bream (initial body weight = 3.8 ± 0.1 g) were randomly allocated in 16 tanks (500 L) to create quadruplicates of four dietary treatments: control group (UP0, 0 % UP), 25 % fishmeal replacement (UP25, 4.4 % UP inclusion), 50 % fishmeal replacement (UP50, 8.5 % UP inclusion) and 75 % fishmeal replacement (UP75, 12.6 % UP inclusion). The growth trial lasted for 96 days.

Samples of liver, intestine and skin mucus were collected from the fish after the growth trial to analyse the activity of oxidative stress biomarkers.

**Results and discussion**
Growth remained similar up to UP50, whereas fish fed UP75 treatment presented a lower growth performance ($p<0.05$). These results are in agreement with previous results reporting that protein coming from *Methylococcus capsulatus*, in general, does not disrupt fish growth with a maximum inclusion of 5-10 % in the feed, depending on the species (Carvalho et al., 2023; Ruiz et al., 2023).

The antioxidant response remained stable throughout all the analysed tissues and measured parameters in agreement with similar studies using the same raw material (Ruiz et al., 2023). Other studies have reported an increased antioxidant response when fed *Methylococcus capsulatus* meal (Chama et al., 2021). Further research is needed to deepen the study behind the mode of action of this novel raw material depending on the dose supplemented *vs* basal diet as well as on the fish species.

**Conclusions**
The present research proves that Uniprotein® can successfully replace up to 50 % of fishmeal in a gilthead sea bream diet without impairing growth.

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Table 1. Growth, disease challenge and antioxidant system of gilthead sea bream (*Sparus aurata*) fed with Uniprotein® (UP)

| Growth | UP0 | UP25 | UP50 | UP75 |
|---|---|---|---|---|
| FBW (g) | 85.92 ± 2.67[a] | 86.49 ± 1.47[a] | 83.34 ± 2.19[ab] | 78.03 ± 2.72[b] |
| SGR (%/day) | 3.18 ± 0.02[a] | 3.18 ± 0.02[a] | 3.14 ± 0.04[ab] | 3.08 ± 0.04[b] |
| FCR | 1.46 ± 0.05 | 1.54 ± 0.10 | 1.51 ± 0.06 | 1.53 ± 0.09 |
| Antioxidant system | | | | |
| LPO liver | 0.67 ± 0.26 | 0.60 ± 0.09 | 0.62 ± 0.09 | 0.56 ± 0.16 |
| FRAPs liver | 1793 ± 256 | 1684 ± 350 | 1727 ± 462 | 2028 ± 689 |
| CAT liver | 7.85 ± 1.44 | 6.91 ± 1.64 | 8.07 ± 0.91 | 7.58 ± 1.17 |
| LPO intestine | 0.66 ± 0.19 | 0.63 ± 0.05 | 0.77 ± 0.14 | 0.66 ± 0.04 |
| FRAPs intest | 1201 ± 395 | 1027 ± 268 | 1149 ± 142 | 1362 ± 732 |
| CAT intestine | 5.57 ± 0.72 | 5.28 ± 0.60 | 5.47 ± 0.33 | 5.62 ± 0.94 |
| FRAPs mucus | 41.56 ± 13.65 | 45.82 ± 5.10 | 56.66 ± 7.24 | 52.97 ± 13.24 |

UP: control diet; UP25: 25 % fishmeal replacement; UP50: 50 % fishmeal replacement; UP75: 75 % fishmeal replacement. FBW: final body weight; FI: feed intake; SGR: standard growth rate; FCR: feed conversion ratio; LPO: lipid peroxidation level; FRAPs: ferric reducing antioxidant power of serum; GR: glutathione reductase; CAT: catalase. [a], [b] superscripts show statistical differences ($p < 0.05$).

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# DIETARY MICROALGAE INTAKE ENHANCES ANTIMICROBIALS ACTIVITIES OF JUVENILE GILTHEAD SEABREAM

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## Introduction

One of the main challenges in modern aquaculture is the control of infectious diseases, which are the leading causes of mortality in farmed fish, particularly during the juvenile stages. These losses not only reduce productivity but also lead to a reliance on pharmaceutical treatments, such as antibiotics, whose use raises serious environmental and health concerns, including the emergence of antibiotic-resistant bacterial strains. In recent years, interest on immunostimulation strategies has increased. In this context, microalgae have emerged as promising ingredients in aquaculture diets due to their richness in bioactive compounds such as polysaccharides, carotenoids, polyunsaturated fatty acids (PUFAs), and natural antioxidants with demonstrated immunomodulatory effects (Matin et al., 2024). Previous studies have shown that the inclusion of microalgae in diets for marine species such as gilthead seabream (Sparus aurata) can stimulate parameters of the innate immunity, such as peroxidase and lysozyme activities (Chaves-Pozo et al., 2014). Incorporating microalgae into fish feed represents a sustainable and effective strategy to strengthen the immune response and reduce disease incidence, thereby contributing to improved animal welfare and the efficiency of intensive aquaculture systems. However, several studies have indicated that this is not always reflected in significant improvements in fish physiological condition (Cerezuela et al., 2012). This discrepancy may be due to the presence of a robust cell wall in some microalgae, which acts as a physical barrier that reduces the bioavailability of intracellular compounds for carnivorous fish (Wu and Tian, 2017; Yong et al., 2020). For all these reasons, it is necessary to study whether the introduction of hydrolysed microalgae improves the availability of immunostimulant compounds contained within the cells.

## Materials and Methods

An experimental trial was conducted at the aquaculture facilities of the Oceanographic Center of Murcia (COMU-IEO), CSIC using juvenile gilthead seabream (*Sparus aurata*), with an initial average weight of 25±0.5 g. The fish were randomly distributed in 15 tanks. During 99 days, the fish were fed five different diets: a control diet (C) and four experimental diets including a mix of algal biomass plus the macroalga *Alaria esculenta* at two inclusion levels (5% or 10%), either in raw form (groups R5 and R10) or previously hydrolyzed with cellulase (groups H5 and H10). Experimental groups were established in triplicates tanks. Samples were taken at days 45 and 99 post-treatment (dpt), and bactericidal and peroxidase activities and the levels of different antimicrobial peptides (hepcidin, lysozyme, and Nk-lysin) in the head kidney and serum were evaluated. The bactericidal activity was measured by the inhibition of the growth of a standard culture of the bacterium *Vibrio harveyi*, following the protocol described in (Chaves-Pozo et al., 2019; Sunyer et al., 1995)"ISSN":"00448486","abstract":"The shi drum (Umbrina cirrosa, peroxidase activity was analysed as an indicator of the non-specific oxidative response (Quade and Roth, 1997) and the quantification of hepcidin, NK-lysin and Lysozime (antimicrobial peptides, AMPs) was performed using an indirect enzyme-linked immunosorbent assay (ELISA) (Cervera et al., 2022).

## Results

Serum bactericidal activity showed a significant decrease in all groups at 45 dpt, as well as in the groups supplemented with raw microalgae at 99 dpt. Regarding serum peroxidase activity, a significant increase was observed in the groups fed with raw microalgae at 45 dpt, and in group H10 at 99 dpt.

Several antimicrobial peptides levels in serum were assessed; however, they remained undetectable due to being below the sensitivity threshold of the analytical method used. In contrast, we could detect them using head-kidney homogenates. Thus, at 45 dpt, a significant increase in the levels of antimicrobial peptides was observed. Specifically, elevated levels of Nk-lysin and hepcidin were detected in group H5, and increased lysozyme levels in groups R5 and H10. However, at 99 dpt no significant differences were found between groups in hepcidin levels, although significant increases in Nk-lysin were still observed in group H5, and in lysozyme in groups R10, H5, and H10.

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**Discussion**

The data obtained indicate that the inclusion of raw microalgae in the diet of fish induces a favourable modulation of the innate immune response. It has been reported that the dietary inclusion of crude microalgae could modulate slightly protease activity, peroxidase and antibacterial activities (Chaves-Pozo et al., 2014). This modulation is reflected in the increased serum peroxidase activity and elevated levels of antimicrobial peptides such as NK-lysin, hepcidin, and lysozyme in the head kidney tissue, especially at 45 days post-treatment. Although a reduction in serum bactericidal activity was observed during the same period, this phenomenon could be interpreted as a homeostatic response aimed at preventing inflammatory overactivation (Soliman and Barreda, 2023).

On the other hand, the elevated levels of NK-lysin and lysozyme observed up to 99 days post-treatment suggest a prolonged immunomodulatory effect of microalgae supplementation. These antimicrobial peptides are key components of the immune response in fish, responsible for the direct destruction of pathogens and the regulation of inflammation (Wang et al., 2017).

Therefore, dietary supplementation with raw microalgae in *Sparus aurata* induces significant modulation of the immune response, evidenced by sustained increases in peroxidase activity and the expression of key antimicrobial peptides (NK-lysin, hepcidin, and lysozyme). These findings suggest a prolonged immunomodulatory effect that could enhance pathogen resistance and improve the organism's immune status.

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# IMMUNOMODULATORY EFFECT OF A MICROALGAE ENRICHED DIET ON *Sparus aurata* LARVAE

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## Introduction

Nutrition during the larval stage of fish represents one of the main challenges in modern aquaculture due to the high natural mortality rates and the need for highly digestible and balanced diets. In this context, the use of microalgae as functional feed ingredients has gained increasing interest, not only because of their nutritional profile, rich in essential fatty acids, proteins, and pigments, but also due to their potential to enhance growth, survival, and the health of developing fish (Reitan et al., 1997). Various microalgae generous, such as *Nannochloropsis*, *Isochrysis*, and *Tetraselmis*, have been incorporated into experimental feed formulations, showing promising results in various marine and freshwater fish species (Ayala et al., 2020). It has been known that blending microalgae species in animal feed is worthy to provide better balanced nutrition to animals (Sarker et al., 2020), however, it is still necessary to optimize their inclusion in order to maximize the nutritional and functional benefits in fish larval diets.

## Materials and Methods

An experiment was conducted at the aquaculture facilities of the Oceanographic Center of Murcia (COMU-IEO), CSIC, with gilthead seabream (*Sparus aurata*) larvae of 0.034±0.005 mean weight grams. Larvae were randomly distributed in 15 tanks and 5 experimental groups (3 tanks/group) were performed to feed with 5 different diets. A control diet free of algae was used, along with four diets containing mixtures of microalgae (*Arthrospira*, *Chlorella*, *Desmodesmus*, *Tisochrysis*, *Nannochloropsis*, *Scenedesmus*, *Tetraselmis*), either in raw (R) or hydrolyzed (H) form, at inclusion levels of 5% and 10% (R5, R10, H5, H10). Larvae were fed ad libitum for 44 days with the aforementioned diets. Sampling was conducted at day 21 and 44 post-treatment (dpt). The fish were then fed with a commercial diet for a year, followed by an additional sampling (365 dpt). Bactericidal and peroxidase activities as well as the levels of expression of immune-related genes were evaluated in whole larvae at days 21 and 44 post-treatment and in fish serum collected at 365 dpt.

Bactericidal activity was determined by the inhibition of bacterial growth of a *Vibrio harveyi* cultures, following protocols described in (Chaves-Pozo et al., 2019; Sunyer et al., 1995)”ISSN”:”00448486”,”abstract”:”The shi drum (Umbrina cirrosa, the peroxidase activity was analysed as an indicator of the non-specific oxidative response (Quade & Roth, 1997), and immune-related gene expression by real-time PCR (qPCR) (Cervera et al., 2023) was used for characterizing the immunomodulatory role of microalgae. Statistical analyses (ANOVA, followed by Tukey’s post hoc or Student’s T tests ($P \leq 0.05$)) were performed using Statgraphics Centurion 15.2.06 version.

## Results and discussion

The results showed that supplementation with microalgae, both in its raw and hydrolyzed forms, had modulatory effects on various immunological parameters and gene expression patterns over time. Bactericidal activity decreased at 21 dpt in all experimental groups, which may reflect an attenuated initial immune response. Although peroxidase activity showed no significant differences between groups at 21 dpt, a general decrease was observed at 44 dpt, except in the H10 group, where it remained comparable to control levels. In the long term (365 dpt), it showed a significant increase, suggesting a sustained immunostimulatory effect of the hydrolyzed microalgae at this concentration.

Regarding gene expression, an early downregulation (21 dpt) of key genes related to innate immunity (*nkl* and *lyz*) and autophagy (*lamp2*) was detected, which could indicate an adaptation or initial modulation phase of the immune system. However, an up-regulation of the *hsc70* gene was observed in the R5 group, pointing to a compensatory stress. At 44 dpt, the R10 group showed an immune activation, reflected by the overexpression of *nkl, ulk1a, and cd8* genes, suggesting a reactivation of the cytotoxic immune response and autophagy mechanisms.

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All together, these findings indicate that the immunomodulatory effects of microalgae depend on multiple issues, the form of processing, the concentration or the duration of exposure, showing biphasic effects: an initial suppression followed by immune activation or recovery in later phases.

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# AGE DETERMINATION AND GROWTH OF NILE TILAPIA (*Oreochromis niloticus*) IN LAKE HAYQ, ETHIOPIA

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Age and growth of Nile tilapia, *Oreochromis niloticus* from Lake Hayq were studied from June 8, 2022 to June 7, 2023. A total of 738 otoliths were collected from the shore, open water and river mouth sites of the lake for otolith macrozone analysis. Otoliths were ground and examined under a dissecting microscope at 7–40X magnification for the presence of translucent and opaque macrozones using a reflected light source. The age of the fish was determined from the total number of translucent macrozones counted in the otoliths considering the time of translucent zone formation. The collected samples ranged from 9.3 to 32 cm in total length (TL) and from 14 to 495 g in total weight (TW). However of the fish sampled, 87.7 % were ≤ 13 cm. The maximum life span of *O. niloticus* was 6 years. A strong relationship between otolith length and otolith weight ($r^2 > 0.88$) was found in *O. niloticus*. The relationships between fish length and otolith (length) were ($r^2 > 0.92$). In *O. niloticus*, otolith weight was found to be best correlated with fish weight ($r^2 > 0.81$) and nearly the same correlation was discovered between otolith length and otolith weight. *Oreochromis niloticus* caught in Lake Hayq has asymptotic length ($L\infty = 31$ cm) and have a growth rate (k) 0.90/year, growth performance index (Ø'L =2.93 and scientific information related to growth parameters is one of the fundamental factors for population management in an aquatic area.

# GROWTH PERFORMANCE OF COMMON CARP (*Cyprinus carpio*) FED A DIET BASED ON CONVERSION OF FISH SLUDGE INTO INSECT MEAL AND CO-CULTURED WITH LETTUCE (*Lactuca sativa*) IN A ONE-LOOP LABORATORY AQUAPONIC SYSTEM

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**Introduction**

Aquaponics, as an integrated system combining aquaculture and hydroponics, has emerged as a promising solution for sustainable food production, particul Insects provide a promising means to bioconvert organic wastes and side-streams into a protein-rich biomass. In this context, the common carp (*C. carpio*), a resilient and extensively cultivated freshwater species, can be efficiently co-cultivated with leafy vegetables such as lettuce (*L. sativa*), which is distinguished by its abbreviated growth cycle and elevated market value. In aquaponic systems, a considerable amount of solid wastes (fish sludge) is accumulated, the management of which has become a significant issue. The disposal of these water pollutants, containing high levels of nitrogen and phosphorus, poses a serious environmental challenge. Similarly, hydroponic and greenhouse cultivation generate residues in the form of leaves, stems and discarded vegetables, which are mostly wasted. Therefore, effective waste management solutions are necessary for both wastes. One potential approach to address this issue is the upcycling of these non-value organic wastes by using insects as bioconverters.

In addition, the utilization of a treated local municipal wastewater that is able to be used again as a reclaimed water and as a water source for aquaponics is a challenge that will produce fish and vegetables locally with zero environmental foodprint. It combines the circular economy approach and the nutrient recycling and water reuse. Based on the above, in the framework of the AWARE project the performance of larvae of the black solider fly, *Hermatia illucens*, fed with wheat bran-based substrates supplemented with various percentages of fish sludge and hydroponic by-products was evaluated. The aim of the present study was to investigate the growth performance of common carp (*C. carpio*) fed a diet based on conversion of fish sludge into insect meal co-cultivated with lettuce (*L. sativa*), in a laboratory scale one-loop aquaponics system using reclaimed water from the local wastewater treatment plant.

Table 1: Growth performace indicators of common carp co-cultures with lettuce.

| | **W0** | **W60** | **W100** |
|---|---|---|---|
| **Initial Body Weight (g)** | 2.00±0.07$^{a}$ | 1.98±0.06 $^{a}$ | 2.02±0.07 $^{a}$ |
| **Body Weight day 15 (g)** | 2.64±0.08$^{a}$ | 2.97±0.08$^{b}$ | 2.70±0.12$^{b}$ |
| **S.G.R. (%/day)** | 0.62±0.09$^{a}$ | 0.96±0.08$^{b}$ | 0.68±0.12$^{a}$ |
| **Initial Plant Weight (g)** | 3.91±0.07 $^{a}$ | 3.90±0.08 $^{a}$ | 3.91±0.09 $^{a}$ |
| **Initial Plant Height (cm)** | 9.62±0.09 $^{a}$ | 9.61±0.09 $^{a}$ | 9.59±0.12 $^{a}$ |
| **Plant Height day 10 (cm)** | 13.87±0.27$^{a}$ | 14.11±0.83$^{a}$ | 14.47±0.30$^{a}$ |

Data were expressed as mean ± S.E.M ($n_{fish}$ = 180, $n_{plant}$= 45). Different superscript letters within rows indicate significant differences (ANOVA, $p$ >0.05).

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**Materials and Methods**

The experiment was conducted at the Aquaculture Laboratory of the University of Thessaly (EL-43BIO/exp-01), approved by the Ethics Committee of the Region of Thessaly (approval no. 112841/23-03-2022). Three one-loop recirculating aquaponic systems (335 L each) were used, each comprising of three fish tanks (50 L), three hydroponic subsystems for plant cultivation (50 L), and one sump filter (35 L). The aquaponics systems were divided into three treatments as follows: the control treatment, which was used 100% freshwater (W0), in contrast to the other two treatments, which were using reclaimed water as follows: 100% reclaimed water (treatment C-W100), and treatment B, which consisted of 60% reclaimed water and 40% freshwater (W60). A total number of 180 common carp (*Cyprinus carpio*), with initial weight of 2.01 ± 0.04 g and 45 lettuce plants (*Lactuca sativa*), with initial weight of 3.91 ± 0.05 g were used. The experiment lasted 75 days. During the experiment, the fish were fed *ad-libitum* by hand twice daily (09:00 and 17:00 with an experimental diet (1.2 mm) (crude protein 52%, crude fat 13.2 %, energy 6.2%). The experimental fish diet was based on insect meal that was produced by insect larvae that were grown on fish sludge and hydroponic by-products. The fish were reweighed every two weeks, while the plant height was remeasured every 10 days throughout the experiment. The fish tanks were cleaned and siphoned daily. At the end of the experiment, the fish were anaesthetize with 0.20 mg/L MS-222, and their final body weight and length were measured. Oxygen levels were maintained at 85%-90% saturation.

**Results and Discussion**

At the beginning of the experiment, there were no significant differences between the initial weight and length of the fish and the initial weight and height of the lettuce in all treatments (Table 1, $p>0.05$). The mean weight of the common carp on day 15, as well as SGR15d, showed statistically significant differences between treatments (Table 1, $p<0.05$). The maximum value was observed in the W60 treatment. The initial mean carp body length and mean carp weight at day 15 used in the present study (4.81 ± 0.03 cm) are in agreement with the results reported in Stamelos et al. al., (2024).

AWARE research project assessed the growth performance and bioconversion efficiency of larvae of the insects fed fish waste- and crop residues-based diets. Specifically, feeding substrates containing wheat bran with different percentages of aquaponic byproducts, i.e., fish faeces and hydroponic plant residues, were evaluated as feed for *H. illucens* larvae. The results showed that most diets containing aquaponic byproducts efficiently supported larval growth, indicating that insect farming could provide a sustainable solution for waste management in aquaponic systems. When fish sludge or hydroponic by-products were included in the diet at <50% rates , no significant differences were recorded compared to the wheat bran control.

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# EFFECTS OF SATIATION DAILY FEEDING SUPPLY AND FOOD DEPRIVATION ON AMMONIA MASS BALANCE IN A BRACKISH POLYCULTURE AQUAPONICS SYSTEM

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## Introduction

The need for Integrated Multi-Trophic Aquaponics (IMTA) systems, especially in brackish water environments, has been highlighted by the growing demand for sustainable food production and the environmental challenges associated with intensive aquaculture. Euryhaline fish species like European seabass (*Dicentrarchus labrax*) can be co-cultivated with halophytes like rock samphire (*Crithmum maritimum*) to enhance nutrient recycling and water quality through natural biofiltration (Vlahos et al., 2023). Ammonia, the primary nitrogenous waste excreted by fish, plays a main role in system sustainability. It is influenced by nutrient inputs, salinity and feeding regimes (Stathopoulou et al., 2021). The efficient removal and conversion of ammonia through plant uptake and microbial nitrification are key components of the functionality of an aquaponic system. Previous studies have demonstrated that high fish and plant productivity can be achieved in brackish aquaponics systems, while reducing the nitrogenous waste load (Pinho et al. 2017). The aim of the present study was to investigate and evaluate the ammonia mass balance in a polyculture brackish aquaponics system produced by gilthead seabream (*Sparus aurata*) and sea bass (*D. labrax*) as an impact of daily food intake and fasting schedule conditions co-cultuvated with glasswort.

## Materials and Methods

The experiment was conducted at the Aquaculture Laboratory of the University of Thessaly (experimental unit EL-43BIO/exp-01), approved by the Ethics Committee of the Region of Thessaly (approval no. 112841/23-03-2022). The rearing system consisted of nine autonomous aquaponic recirculation systems with a total volume of 135 L; each consists of 45 L fish tanks, a 45 L hydroponic subsystem for plant culture and a 45 L sump-type filter. The water turnover rate was calculated to be four times per hour (Stathopoulou et al. 2021). In totaly, were used 90 individuals of *S. aurata* with average body weight and length of 6.33 ±0.73 g and 5.73±0.72 cm, and 90 individuals of *D. labrax* with an average body weight of 5.82±0.77 g and average body length of 6.35± 0.45 cm. The experiment lasted 75 days.

**Table 1**. Filter performance and ammonia balance in the three aquarium systems under treatment A, B, and C throughout the experimental procedure.

| | S0 | S4 | S7 |
|---|---|---|---|
| Filter efficiency (E) | $0.91\pm0.53^{a}$ | $1.08 \pm 0.49^{a}$ | $0.65\pm0.33^{a}$ |
| Ammonia oxidation rate (AAR, g/m2/day) | $0.09 \pm 0.04^{a}$ | $0.08 \pm 0.02^{a}$ | $0.06 \pm 0.02^{a}$ |
| Volumetric Ammonia Oxidation Rate (VTR, $g_{TAN}/m^3/d$) | $4.15 \pm 0.79^{a}$ | $5.43 \pm 0.47^{b}$ | $4.64 \pm 0.93^{a}$ |
| Ammonia inlet, (Q*CTANin) + PTAN | $15.51\pm1.34^{a}$ | $11.32\pm0.59^{b}$ | $12.80\pm0.16^{b}$ |
| Ammonia outlet, (Q*CTANout) + RTAN | $10.03\pm1.55^{a}$ | $19.19\pm1.09^{b}$ | $15.52\pm1.39^{b}$ |

Data were expressed as mean ± S.E.M (n = 75). Different superscript letters within rows indicate significant differences (ANOVA, p >0.05).

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Food intake was *at libitum* three times per day (Grigorakis & Alexis, 2005) by hand at 09:00 h, 13:00 h, and 17:00 h. with a formulated diet (1,2 mm) (crude protein 43.7 %, crude fat 15.3 %, energy 23.2 Mj/kg). The three aquaponics systems were devided into three treatments according to Ziegelbecker & Sefc (2021) as follows: daily feed intake (S0 control treatment), a 4-day deprivation (S4 treatment, 3 days feeding and 4 days starvation) and a 7-day deprivation (S7 treatment, 7 days feeding and 7 days starvation). The fish were weighed every two weeks, while the plant height was measured every 10 days throughout the experiment.The fish were reweighed every two weeks, while the plant height was remeasured every 10 days throughout the experiment. The fish tanks were cleaned and siphoned daily. Fish growth indicators were measured using the following equations: SGR (%/day)= [(ln Wfin-lnWin) /Δt]x100.Ammonia mass balance was measured from the equation (Q*CTANin) + PTAN= (Q*CTAN**N**out) + RTAN, Volumetric Ammonia Oxidation Rate from the equation: (VTR, $g_{TAN}/m^3/d$)= (TANin – TANout) * Kc * Q/V, and filter efficiency was measured according toy the equation: **E** =[1+(C*R)-C]/C*R.

**Results and Discussions**

There were no statistically significant differences in filter efficiency, ammonia oxidation rate or volumetric ammonia rate in both aquaponics treatments (ANOVA, $p>0.05$). However, total ammonia levels in the influent and effluent of the aquaponic systems showed significant differences between treatments (ANOVA, $p<0.05$), with the highest values in treatment S0 in terms of TAN in and S4 in terms of TAN out (Table 1).

The filter efficiency (E) in the experiment was 0.91 ± 0.53 in treatment S0, 1.08 ± 0.49 in S4 and 0.65 ± 0.33 in S7and is influenced by the ammonia production rate, the ammonia removal rate, fish and planta species, food composition, and rearing conditions (Pinho et al., 2017). Ammonia production rate (AAR) was 0.09 ± 0.04 mg/L for treatment S0, 0.08 ± 0.02 mg/L for treatment S4 and 0.06 ± 0.02 mg/L for treatments S7, which was higher than those reported by Vlahos et al. (2023) which was ranged from 2.79 ± 0.34 to 3.03 ± 0.45 mg/L. Our results showed a range of filter volumetric oxidation rate (VTR, g TAN/m3/d) values from 4.15 ± 0.79 g TAN/m3/d to 5.43 ± 0.47 g TAN/m3/d. The VTR values in the present experiment are in agreement with those (6.44 ± 1.69 g TAN/m3/d) reported by Vlahos et al. (2023) in a study that involved rearing *D.labrax* and cultivating *C. maritimum* in an aquaponic system with water recirculation.

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# HYPOXIA-INDUCED IMMUNE, METABOLIC AND REDOX CHANGES IN ATLANTIC SALMON GILLS

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## Introduction

Climate change-driven reductions in dissolved oxygen have led to widespread environmental hypoxia, a primary cause of mortality in salmonid aquaculture (Soto et al., 2019). The gills, which are essential for both gas exchange and immunity (Olaf Koppang et al., 2015. This tissue are highly responsive to low oxygen environment, wich induces oxidative strees and modulate key signaling pathways (Wang *et al.*, 2023). Therefore, understanding the immunological, metabolic, and antioxidant responses to different hypoxia regimes is crucial for optimizing the resilience of *Salmo salar* in aquaculture.

## Methodology

*Salmo salar* individuals were exposed to three conditions: (1) continuous hypoxia at 45% $O_2$ saturation (45% $DO_2$), (2) continuous hypoxia at 75% $DO_2$, and (3) cyclic hypoxia (12 h 50% $DO_2$ / 12 h normoxia). Gill samples were collected on days 1, 3, 5, and 7. We performed microarray sequencing analysis to identify differentially expressed genes in hypoxic conditions (log fold change |±2|) and qPCR analysis was conducted for genes associated with hypoxic response (HIF1α, EGLN, VEGF, EPO), immunity (TNFα, IL1β, IL8, TLR3, TLR5, TLR9, NOD1, NLRX1, NLRC3, NLRC5), and antioxidant activity (GSH-Px, SOD, GSH-Rx).

## Results

Our transcriptomic analysis revealed that the intensity and pattern of hypoxia determined distinct molecular responses in the gills of *S. salar*. At 45% $DO_2$, a sustained activation of immune and cellular stress responses was observed, characterized by early overexpression of mucin and mitochondrial proteins along with ubiquitins on day 1, followed by a progressive increase in cathelicidin from days 3 to 7. Furthermore, Myelin and lymphocyte protein (MAL) gene remained overexpressed throughout the experiment, which has been associate to epithelial remodeling.

At 75% $DO_2$, the response was faster and transient: mucin, aquaporins, and MAL were overexpressed on day 1, while cathelicidin was overexpressed on day 5, with no sustained changes thereafter. In cyclic hypoxia, the overexpression of mucin and complement component C3 genes were mainly on day 1, suggesting a damage response.

Complementary qPCR analysis confirmed these findings: at 45% $DO_2$, there was a sustained increase in *tnfα* (days 1, 5, and 7) and *phd3* (*egln*) (days 1 and 3), decreased *hif1α* (day 5), and reduced expression of antioxidant enzymes such as *gsh-rx* (day 1) and *gsh-px* (day 5), along with decreased expression in *tlr3* (day 3). At 75% $DO_2$, *hif1α* increased on days 1 and 5, accompanied by early increases in *gsh-rx* and *sod* genes, as well as *tnfα* and *il1β*, but with a marked reduction of tlr9 on all days and punctual decreases in *tlr3* and *tlr5*. In cyclic hypoxia, an initial increase in *phd3* (*egln*) (day 1) and *epo* (day 3) was observed, along with decreases in *gsh-rx* and *sod* (day 5) and *nlrc3* genes (day 7).

## Discussion and Conclusion

The combination of transcriptomic and qPCR data demonstrates that hypoxia modulates immunity, metabolism, and redox status in *S. salar* gills in a complex manner. Recent studies showed that chronic hypoxia in *S. salar* can differentially modulate the immune response in the head kidney (Rojas *et al*., 2025). The 45% $DO_2$ condition generated severe and persistent stress, activating innate immune pathways, epithelial repair mechanisms, and metabolic adjustments. At 75% $O_2$, the response was less intense and more transient, although the sustained reduction in *tlr9* gene suggests a potential vulnerability in pathogen recognition. Cyclic hypoxia induced an immediate and short-lived response, aimed at the rapid activation of physical barriers and complement, along with punctual alterations in antioxidant genes.

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In conclusion, these results showed that salmon adaptation to hypoxia is not uniform but depends on the intensity and pattern of exposure, involving dynamic interactions between physical barriers, innate immunity, metabolic regulation, and antioxidant systems. The identification of these pathways opens opportunities for management strategies and genetic selection to increase hypoxia tolerance and maintain immune functionality in aquaculture environments at risk of low oxygen availability.

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# MINING *Lepeophtheirus salmonis* TRANSCRIPTOMES UNEARTHS KEYS TO STAGE-SPECIFIC QUNTIFICATION OF PARASITIC LARVAE

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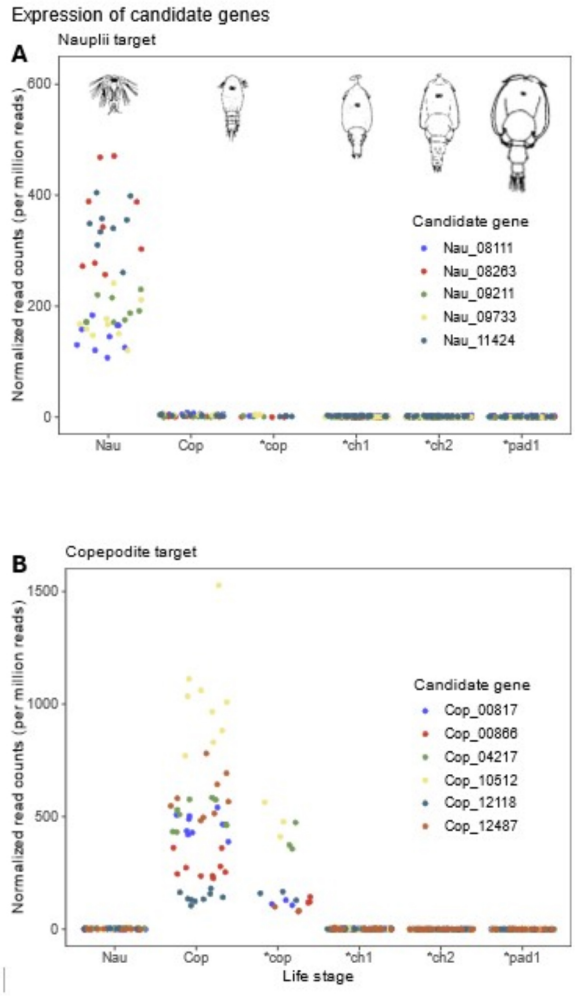
The salmon louse, *Lepeophtheirus salmonis,* is an ectoparasitic copepod which has devastating effects on both wild and farmed salmonids in the North Atlantic region. To better assess risk to wild salmonid populations there is an urgent need to individually quantify the different planktonic life stages of *L. salmonis*. Current molecular methods show great promise reliably identifying and counting *L. salmonis* individuals but are unable to discern between the infective and non-infective larval stage. In the present study, comparative transcriptomics of nauplii and copepodid larval stages was used to identify transcript candidates for stage-specific quantification of *L. salmonis* larvae. We demonstrate that approximately two thirds of *the L. salmonis* transcriptome is differentially expressed between the two planktonic life stages. We present 11 candidate quantitative PCR assays targeting RNA transcripts expressed solely in one of these two life stages, of which four were tested *in vitro*. All four candidates successfully amplified target life stage RNAs with high detection rates, of which three also showed capability as assays for separating the life stages of *L. salmonis* and measuring their densities using quantitative PCR. Applying these assays to well established eDNA collection and processing pipelines could pave the way to significantly upscale lice monitoring practices in regions with high infestation pressure or at-risk salmonid populations.




Two dot plots show normalized gene expression of the 11 candidate genes split into the nauplii(A) and copepodite(B) larval life stages of L. salmonis. Candidate genes are highly expressed in RNAseq samples containing their respective target life stage while non-target life stages have expression at or near zero for all candidate genes. The left two columns in each plot show read counts generated from the nauplii and copepodite samples in this study while the right four columns show expression values extracted from supplemental tables published by Eichner et al. (2018) for copepodite, chalimus 1 and 2, and preadult 1 stages samples. Drawings of the life stage are adapted from Schram (1993).

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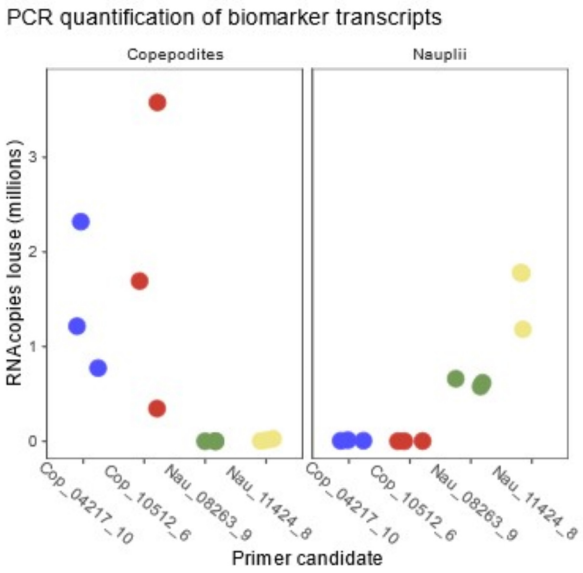


Dot plots showing transcript quantification via ddPCR for triplicate samples of L. salmonis nauplii and copepodites in densities of 10 larval individuals. A high density of transcripts were detected by the assays for each of the target life stages. All non-target life stage samples generated transcript detections near-zero. Copepodite samples showed higher variability of stage-specific RNA abundance across the three sample replicates.

# HARNESSING CURRENTS TO QUANTIFY PARASITES: MOLECULAR ANALYSIS OF PASSIVELY COLLECTED PLANKTON PARTIALLY VALIDATES A SALMON LICE DISPERSAL MODEL

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Modeling the dispersion of marine species and debris is a critical technological development that has aided fisheries management, aquaculture development, and marine conservation. In Norway, a hydrodynamic particle dispersion model (HPDM) is applied to salmon lice larvae, the planktonic life stages of the ectoparasitic copepod that infests salmonid fishes. These models produce estimated densities of infective larvae along the Norwegian coast which are used to assess risk of infestation of migrating wild salmonids and plan lice prevention strategies at salmon farms. Because the risk assessments generated by these models impact industry regulation and environmental conservation efforts, their predictions in some regions are hotly contested and the capability to provide spot verifications would be beneficial for settling disputes. Based on this need, we designed a method for passively filtering tens of thousands of liters of near-surface seawater to capture planktonic organisms for preservation and downstream analysis by ddPCR eDNA quantification. We deployed 12 of these devices in the Hardangerfjord in western Norway, where surveys of louse infestation risk have been conducted for over 20 consecutive years and the oceanographic data is collected in verification of norkyst800, one of the hydrodynamic models by which salmon louse dispersion is predicted.

Comparing the number of lice larvae we captured with modeled estimates of louse density and the number lice infesting experimental salmon smolts, we found that spatial and temporal patterns of louse distribution were fairly consistent across methods and there was a high correlation between them. Examining the differences between sites, we observed that those with a high density of farms in the vicinity had a significantly greater abundance of lice captured by passive collection than those without, particularly the one site immediately down current of an active farm. At the one site located in a National Salmon Fjord, an area closed to fish farming, both the model and passive sampler failed to predict / detect the occurrence of parasite larvae, but the experimental smolts at that site became infested with low numbers of louse during both two week cage deployments. Overall, our comparisons show that passive collection methods function effectively to generate substantiated larval lice abundances, and deploying this approach on a large scale could provide general verification of the accuracy of model estimates and also present areas where specific inaccuracies in the model need refinement.

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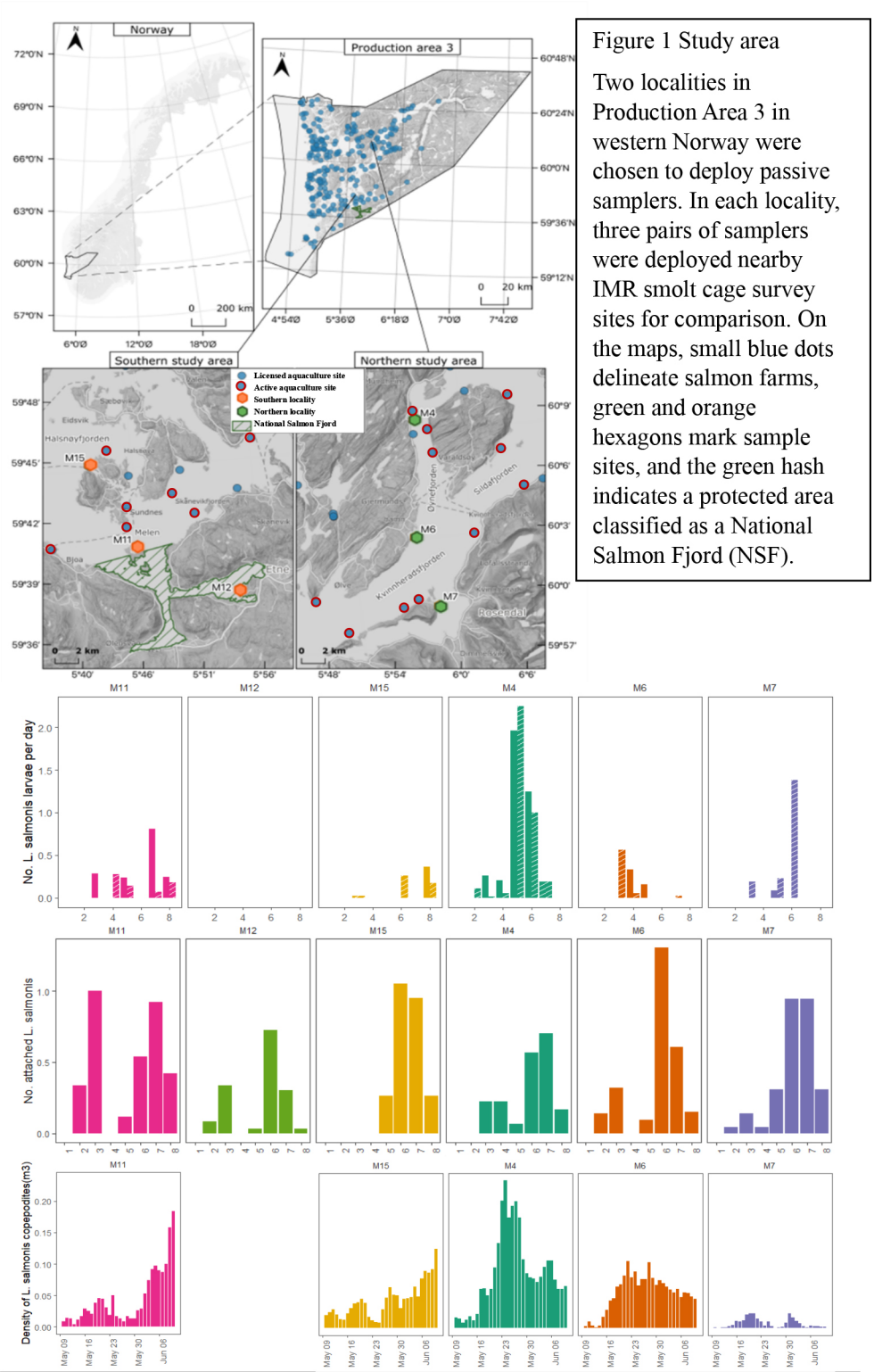


Figure 1 Study area

Two localities in Production Area 3 in western Norway were chosen to deploy passive samplers. In each locality, three pairs of samplers were deployed nearby IMR smolt cage survey sites for comparison. On the maps, small blue dots delineate salmon farms, green and orange hexagons mark sample sites, and the green hash indicates a protected area classified as a National Salmon Fjord (NSF).

Figure 2 Captured / observed / predicted salmon lice larvae from three methods

Bar graphs in the first row show the number of lice captured in each sampler during the eight collection events. Paired samplers at each site are displayed as solid vs patterned bars. Bar graphs in the second row show number of lice attached to experimental fish placed in cages in the fjord. Bar graphs in the third row show daily estimates of lice density as predicted by IMR's salmon louse HPDM. At site M12, inside the NSF, we did not capture any salmon lice and IMR's model was unable to generate louse density estimates.

# DYNAMIC OF THE GUT MICROBIOTA IN RAINBOW TROUT (*Oncorhynchus mykiss*) FED A HIGH CARBOHYDRATE-TO-PROTEIN RATIO DIET

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## Introduction

Farmed carnivorous fish have traditionally required high-protein diets, primarily based on fishmeal (FM) and fish oil (FO) derived from wild marine sources. To ensure sustainable development of the aquaculture sector, alternative protein and energy sources are needed to replace FM and FO by renewable, eco-friendly and sustainable feed ingredients such as digestible carbohydrates (CHOs) (Yang, Ai, and Mai 2025). However, carnivorous fish like rainbow trout are considered as poor utilizers of dietary CHOs, often exhibiting metabolic disorders such as persistent hyperglycemia and hepatic dysfunction over 20% incorporation (Kostyniuk et al., 2019). Despite multiple hypotheses, mechanisms underlying this limited CHO utilization remain unclear (Polakof & Panserat, 2016). The intestine, as the first organ exposed to nutrients, plays a key role in mediating interactions between diet, environment, and host physiology (Polakof et al., 2010). Yet, the involvement of gut microbiota in CHO metabolism in fish remains underexplored. This study aimed to investigate the early dynamics of gut microbiota establishment in rainbow trout fed a high carbohydrate-to-protein ratio diet, to better understand carbohydrate utilization in this economically important species.

## Materials and methods

Following a 4-day feed deprivation period, juvenile rainbow trout were reared for 22 days and fed one of two isolipidic and isoenergetic experimental diets: a high-protein, no-carbohydrate diet (NC; ~60% fishmeal proteins), and a high-carbohydrate diet (HC; 30% digestible carbohydrates and ~40% proteins). Samples ($n$ = 12 per diet) were collected on days 1, 2, 3, 4, 10, 15, and 22. The midgut was opened, and digesta and mucus were separated. DNA was extracted using the QIAamp Fast DNA Stool Mini kit (Qiagen, Hilden, Germany), and the V3–V4 region of the bacterial 16S rRNA gene was amplified. PCR products were sequenced at the sequencing center (Bordeaux, France) using 300-bp paired-end reads. Sequencing data were processed using QIIME 2 (v2024.10), and outputs—including ASVs table, taxonomy, phylogenetic tree, and representative sequences—were exported to the *microeco* R and *mixOmics* R package for further analysis. Body and liver weights, along with plasma glucose concentrations (using the GOD-POD enzymatic colorimetric method), were also measured to assess whether the feeding trial successfully induced distinct nutritional responses. Intestine and hepatic genes expression and proteomics analysis were also addressed.

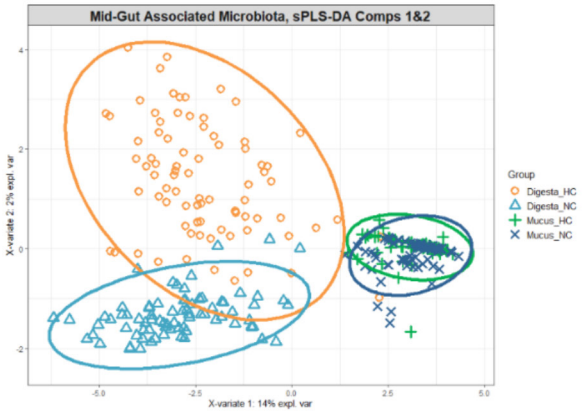


**Figure 1.** Partial Least Squares Discriminant Analysis (PLS-DA) of mid-gut microbiota profiles based on Component 1 and Component 2. Points represent individual samples from mucus- and digesta-associated microbiota under high-carbohydrate (HC) and no-carbohydrate (NC) diets.

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**Results**

Plasma glucose concentrations and hepatosomatic index (HSI) were significantly higher in fish fed the HC diet from day 1. In contrast, no significant differences in body weight were observed between dietary groups throughout the 22-day trial. Across all compartments, diets, and time points, the mid-gut microbiota of rainbow trout was dominated by *Bacillota* (syn. *Tenericutes*) and *Pseudomonadota* (syn. *Proteobacteria*). At genus level, *Malacoplasma* spp. (syn. *Mycoplasma* spp.) were by far the most abundant taxa, averaging approximately 96% in the mucus and 82% in the digesta. The high carbohydrate-to-protein ratio diet significantly affected beta diversity in both digesta- and mucosa-associated communities from day 3. Differential relative abundance analysis, using Random Forest and non-parametric testing, revealed that this dietary shift significantly altered the relative abundance of several genera within the *Lactobacillaceae* family, as well as *Malacoplasma* spp. In contrast, mucosa-associated communities exhibited no major taxonomic shifts suggesting that the mucosa associated microbiota is more resilient to diet changes (Figure 1).

**Conclusions**

A high dietary inclusion of carbohydrates in rainbow trout impacted the dynamics of the gut microbial community, particularly within digesta-associated microbiota when mucosa- associated microbiota is more resilient to diet changes.

**Acknowledgements**

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# OLIVE PULP *Olea Europaea* AS A NATURAL ANTIOXIDANT IMPROVES ASTAXANTHIN RECOVERY AND OXIDATIVE STABILITY IN FISH FEED

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## Introduction

Astaxanthin is a valuable feed ingredient in aquaculture, particularly used in salmonid diets for its pigmentation and health benefits. However, it is highly prone to oxidative degradation during feed processing and storage, leading to color loss and inferior fillet quality, requiring the use of antioxidants. Worldwide regulatory limits on synthetic antioxidants (e.g. BHT, BHA, ethoxyquin) have intensified the need for natural and sustainable alternatives in the dry mix (pre-extrusion) and/or during the fat-coating phase (post-extrusion). Olive oil production residues are rich in potent antioxidants such as polyphenols and triterpenoids. A standardized upcycled olive-pulp was evaluated as potential alternative in feed-preservation programs aimed at protecting astaxanthin and dietary lipids from oxidative degradation.

## Materials and Methods

Pilot-scale extrusion trials were conducted in a three-phase experimental design at the Danish Technological Institute (Denmark). Phase I screened a range of natural antioxidants added to the dry mix against an untreated control and astaxanthin was measured immediately after extrusion. Phase II compared a natural antioxidant program against a conventional synthetic regime. Phase III focused on optimizing the most effective antioxidant programs found in Phase I and II for both the dry mix and the fat coating phases.

All feeds were produced in a pilot-scale twin-screw extruder (Werner & Pfleiderer Continua 37; 35 mm screw diameter, L/D = 24:1, 7.5 kW motor, five-barrel sections). Individual ingredients were milled to <1 mm, weighed, and blended for 15 min in a horizontal ribbon mixer. Each antioxidant test product was hand-premixed with 200 g meal for 30 s to aid dispersion and then incorporated together with synthetic astaxanthin (Carophyll Pink 10 %; 100 mg/kg, DSM).

The mash entered the extruder without live-steam injection; moisture was adjusted by adding 15–17 % water to the feed. Barrel temperatures were set at 103–122 °C; the die-head temperature reached 106–118 °C and the pressure was 15–30 bars. Screw speed was maintained at 60–68 % of rated r.p.m., yielding a torque of 45–54 Nm and a throughput of 40–45 kg/h. Pellets were knife-cut at the die and conveyed to an electric belt dryer (Lytzen; 850 mm belt width, 2.5 m drying zone, 24 kW) operating at 95 °C with a 25 min residence time. Dried pellets were vacuum-coated with 150 g/kg fish oil; diets with a liquid antioxidant were applied via the coating oil.

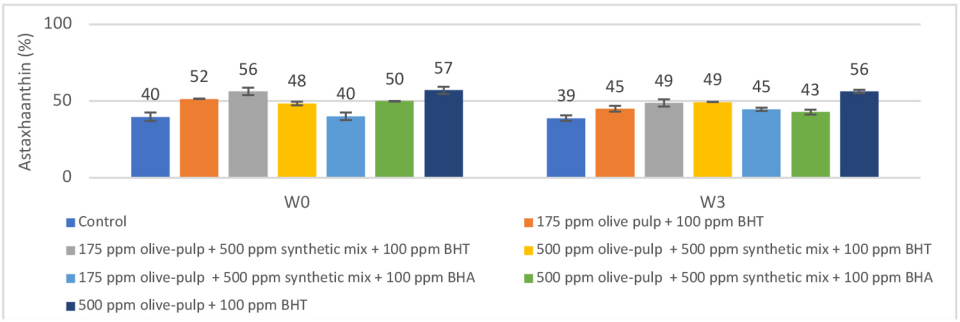


*Figure 1: Astaxanthin recovery (%) in feed after extrusion (W0) and after storage (W3) for the control and six antioxidant programs. Results are reported as mean ± standard deviation of duplicate measurements.*

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In phases I and II, representative samples were collected immediately after extrusion (Week 0 = W0). To simulate an industrial application program, all treatments in phase III, received 100 ppm liquid BHT or BHA (applied each as 500 ppm of a 20% concentration commercial product) in the fat coating phase. Feed samples were collected at W0 W3 upon ambient storage (20-25°C) to test mid-term stability. Selected treatments were stored up to six months (W26) to check long-term stability.

Astaxanthin concentration was determined by HPLC with photodiode-array detection at 474 nm. Primary lipid oxidation was assessed as peroxide value (ferric thiocyanate method, IDF standard) and secondary oxidation as thiobarbituric-acid-reactive substances (TBARS) expressed as mg malondialdehyde/kg feed. All analyses were performed in duplicate.

**Results**

Phase I: In the control feed with no antioxidant, astaxanthin recovery after extrusion was 51% of the formulated level, indicating a 49% loss under the current pilot extrusion conditions. Incorporation of 500 ppm of olive pulp or 500 ppm of a 30% dry tocopherol in the dry mix (i.e. a typical natural antioxidant industrial application) improved astaxanthin retention at equal levels (approx. 60%). Higher dose (1000 ppm) of olive pulp did not yield additional gains, indicating a plateau effect. No differences were observed in lipid oxidation indicators (PV, TBARS) among all treatments.

Phase II: A conventional synthetic antioxidant blend applied in both the dry mix (at 500 ppm, containing 24% BHA, 8% propyl gallate, and 8% citric acid) and the oil coating (500 ppm of the same blend in liquid form, plus 500 ppm of a mix of 10% ascorbyl palmitate and 5% tocopherols) did not offer any additional astaxanthin protection compared to that of 500 ppm olive pulp applied only in the dry mix, as they both resulted in the same astaxanthin retention improvement (approx. 10 % relative to the control feed). Again, no significant differences were found in PV or TBARS between the two treatments.

Phase III: At all time points, inclusion of 500 ppm olive pulp in the dry mix and 100 ppm liquid BHT consistently resulted in the highest astaxanthin recovery % relative to the control feed, without any further astaxanthin losses at least until W3 indicating an excellent mid-term feed stability (Fig.1; W0: 57% vs. 40%; W3: 56% vs. 39%; and, W26: 37% vs. 29% (data not shown)). Other programs containing lower olive doses (175 ppm) and liquid-BHT, or combination of olive pulp with synthetic mixes (17% BHT, 7% propyl gallate and 25% citric acid) in the dry mix and liquid-BHT or liquid-BHA in the fat coating, did not provide any additional advantage to the ‘olive pulp-based program’, retaining anything between 44–50% of astaxanthin after three weeks (Fig. 1). Peroxide values and TBARS showed no differences among treatments, with the exception of the control that’s showed slightly increased values after 3 weeks (data not shown).

**Conclusion**

A pre-extrusion dose of 500 ppm of olive-pulp as a natural antioxidant paired with a minimal (EU-compliant) level of liquid BHT in the fat coating phase, improved significantly the astaxanthin protection in extruded salmon feed. This holds great promise as our results translated to significant reduction in synthetic antioxidant usage without compromising feed stability. Additionally, by preventing astaxanthin depletion, the olive-pulp based program may also safeguard better pigment deposition in the fish itself as it may contribute to improved fillet color and lipid quality. Further testing is required to account for factors such as the diet composition (fat level, presence of blood meal or endogenous antioxidants), extrusion conditions and drying losses.

# CIRCULAR ECONOMY APPROACH IN AQUACULTURE FOR THE REALIZATION OF NEW MARINE INGREDIENTS TO SUPPORT THE GROWING REQUEST FOR MARINE FOOD PRODUCTS

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It is known that the blue sector, and in particular aquaculture, has a crucial role in meet the growing request for seafood and to ensure food security goals. Among the strategies, the valorization of side streams and by-products obtained during the process, for the realization of ingredients, new marine biobased food products, or to extract value added bioactive compounds, is more and more suggested to promote diversification, waste reduction and circular economy. These opportunity targets the SDGs 2030 agenda and the blue transformation strategy suggested by the FAO, that focuses on better productions to achieve a better life.

Within this strategy, the reduction of fish wastes for the production of new food, feed or ingredients, are among the proposed solution to increase the sustainability of the aquatic sector, not only from environmental, but also from economic point of view. Our research group has developed protocols, procedure and technologies that are going to be implemented within the project at regional level, thanks to the strong networking with local stakeholders. We have developed novel marine-biobased ingredients and food from side streams obtained after filleting farmed fish or in integrated multitrophic aquaculture IMTA, such as omega-3 enriched fish oils, protein hydrolizates, antioxidants from extractive species, at high Technology Readiness Level (TRL). These compounds have the potential for the development of new food, feed, pharmaceutical and cosmeceutics, up to cellular agriculture, contributing to environmental, social and economic sustainability, according to the blue transformation strategy.

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# EFFICIENT HANDLING OF TURBOT UNDER INDUSTRIAL PRODUCTION CONDITIONS: HOW LONG AIR EXPOSURE TRIGGERS THE STRESS RESPONSE?

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**Introduction**

Aquaculture is a booming sector due to the growing global demand for seafood. However, intensive fish production requires strictly controlled environments and practices that ensure animal welfare, as a decisive element for the growth and development of farmed fish. Animal welfare has become a key factor in the development of modern aquaculture practices. This concept establishes that animals must be free from any factors that could negatively affect them, whether physically, socially, or psychologically (EFSA, 2009). A crucial aspect of animal welfare is stress reduction. Avoiding stressful situations in aquaculture has been shown to improve fish health and survival, leading to higher farm productivity (Ashley, 2007; Toni et al., 2018). It is therefore important to analyze the stress response due to common practices in aquaculture farms, such as handling of individuals, batch sorting, inadequate stocking density, rearing, slaughter, or transport between farms, in order to improve and optimize them from a welfare perspective.

The aim of this study was to characterize the physiological response to stress caused by exposure to air in turbot, mimicking routine handling practices in land-based aquaculture farms. In addition, an attempt was made to establish a time interval, based on the duration of exposure, in which the welfare of the animals is not significantly compromised.

**Material and methods**

Turbot (*Scophthalmus maximus*, 500–600 g) were acclimated for two weeks under controlled conditions established at the land-based aquaculture farm. After the acclimatization period, groups of fish were caught with a net and exposed to air for 15 seconds, 2 minutes, or 5 minutes, after which they were returned to the water and kept under the conditions indicated above. To evaluate the stress response and its evolution over time, blood samples were obtained at 0.5, 2, 6, and 24 hours after exposure to determine plasma concentrations of stress-related hormones (cortisol and catecholamines), metabolites, and other physiological parameters. The experiments were carried out at the industrial aquaculture facility of Stolt Sea Farm (Ribeira, A Coruña) to replicate real commercial production conditions.

**Results and discussion**

The results obtained in this study suggest that the intensity of the stress response in turbot is directly proportional to the duration of air exposure, with longer exposures resulting in stronger physiological responses. Thus, brief exposure (15 seconds) did not show detectable stress responses compared with the unexposed control group, whereas prolonged exposures (2 and 5 minutes) triggered multiple physiological changes. These variations included reductions in blood gas concentrations, ion levels ($K^+$ and $HCO_3^-$), and pH, along with an increase in plasma glucose levels. These results are consistent with previous studies indicating that air exposure can induce a state of hypoxia, leading to a decrease in oxygen partial pressure and saturation, and a reduction in pH due to lactic acid production as part as a stress defense response (Cook et al., 2015). Prolonged air exposure has been shown to also decrease parameters associated with $CO_2$, likely because of increased opercular activity in response to hypoxia (Gilmour, 2001). As reported in other fish species, plasma glucose levels exhibited a significant increase within the first hours after exposure, reflecting a typical metabolic response mediated by stress hormones such as catecholamines and cortisol (Da Santa Lopes et al., 2023). This hyperglycemic response may, in turn, contribute to the reduction of plasma electrolytes, such as potassium and bicarbonate, to maintain osmotic balance.

Regarding hormonal responses, significant alterations in plasma catecholamine concentrations were only detected in fish exposed to air for 5 minutes. These changes occurred at 0.5 hours post-exposure for noradrenaline and at 6 hours in the case of adrenaline. Cortisol levels in plasma increased significantly within the first hours after prolonged air exposure (2 and 5 minutes), reflecting an activation of the neuroendocrine axis as part of the primary stress response (Pottinger, 2008). All

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hormonal parameters, however, returned to baseline values within six hours post-exposure, suggesting a rapid recovery to control conditions.

Overall, these findings indicate that turbots can withstand brief air exposure without triggering a significant stress response. Consequently, routine aquaculture farming practices involving short-term air exposure (≤ 1 minute) are unlikely to compromise fish welfare in this species.

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# COMPARING THE SUSTAINABILITY OF TECHNOLOGICAL CONCEPTS FOR SALMON-FARMING: OPEN NET-PENS, LAND-BASED, SEMI-CLOSED AT SEA, SUBMERGED PENS

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**Challenges with open net-pens**

The dominant technology for grow-out of Atlantic salmon has for the last fifty years been open net-pens in the ocean. This is thus by now a well-established technology. Still, there are challenges, as pointed out i.a. in the Norwegian Official Report on aquaculture from 2023 (1). This includes related to the spread of parasites and disease, escapees, and emissions of nutrients, particulate matter and hazardous substances. These in turn have negative impacts, for the welfare and mortality of the farmed fish, on biodiversity, and also on other coastal user groups like fishers and recreationists. In particular, the problems with limiting the impacts on and risks for stocks of wild salmonids have led the authorities to limit growth of the industry, and to reduce aquaculture licenses in some Norwegian regions.

**Emerging technological concepts**

To try to reduce or eliminate these problems, several alternative technological concepts are under development, testing and implementation. They include land-based farms, floating semi-closed plants, and submerged pens. Each of them brings promise of dealing with one or more of the challenges that open net pens face. However, they also come with their own challenges and risks compared to open net pens. There have been events with mass-mortality of farmed fish. The concepts require more energy for operations than open net pens. Investment and operational costs are also different, but in less clear ways, as less lice and diseases reduce costs for treatments, and improve growth and survival which have positive effects on the economic feed factor. The new concepts may reduce the original area conflicts, but can also shift these to new areas, whether to land or elsewhere in the ocean.

**Sustainability comparison**

Available information on how the concepts perform, including their impact on the environment and other dimensions of sustainability, varies from sketchy to more reliable. Aquaculture companies that consider investing in a new concept for their plants would benefit from information on different concepts' performance. This goes both for choosing between concepts that are currently available in the market or deciding whether they should wait for a concept under development to become available. The authorities also need to understand the challenges, risks and opportunities with the new technologies. Firstly, they need it to decide on new policies and regulations. Even though the Norwegian government in their recent White paper to the Parliament (2) have underscored technological neutrality for their planned reform of aquaculture policies, they still need to understand what improvements are possible with new technologies in setting targets and plans. Secondly, to decide on actual site licenses with the new technological concepts they need to consider actual negative environmental impacts and risks.

**This presentation**

This presentation has two main elements. The first is a methodological approach to compare and present the sustainability of different technological concepts for aquaculture, and the second is an actual comparison of different technological concepts for the grow-out of salmon.

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We have collected data and information on new technological concepts that have undergone trials, pilots or from actual operations. Although this on a basic level gives more reliable information on how the technologies will perform than for technological that are only on the drawing board, the data from trials still has to be interpreted to consider what can be the performance of the commercial version of the technology in commercial operations. The selection, development and presentation of sustainability indicators is based on the authors' previous experience with this (i.a. 3, 4). The method and graphical tools employed are relevant also for other types of aquaculture, while the actual comparison is highly relevant to the ongoing processes for development and management reform of salmon farming in Norway and also elsewhere, like in Canada.

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# PERCEPTIONS SHAPE SOCIAL ACCEPTANCE FOR AQUACULTURE – BUT ARE PERCEPTIONS SHAPED BY REALITIES?

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**Social acceptance and social license to operate**

The concepts of a *social license to operate* and *social acceptance* for an industry or company and their activities have evolved both in a public context and in academic literature. Originally used within the mining industry, the concepts have also been studied within marine contexts (1), including aquaculture (e.g. 2-3). Methods have been developed to try to measure the level of social acceptance for an industry or company within local communities or for a wider population, and to understand what affects it (4-7).

Studies of mining have found empirical support for persons' perceptions on the distributional justice, procedural fairness, and confidence in governance to significantly affect their trust and their level of social acceptance for an industry or company. However, for aquaculture in Norway, these models have been found to explain the level of social acceptance to a lesser degree (8). Instead, the perceptions of the aquaculture industry's environmental sustainability were found to be the most important factor determining the social acceptance level. Yet, the perceptions on the distribution of benefits from aquaculture were found to be relatively important.

**Perceptions and realities**

It seems people's perceptions to a large degree shape their social acceptance for aquaculture. To what degree people's perceptions are shaped by actual impacts from aquaculture is however less clear. We will present data and statistics on the environmental and socio-economic impacts and conditions of aquaculture in Norway from previous studies and Norwegian datasets we have access to (9-11). This will be combined with data on perceptions and social acceptance from a national survey for Norway (12) into preliminary analyses and hypotheses of how local "realities" affect perceptions and social acceptance.

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# SMALL BLUE MUSSELS FOR SUSTAINABLE PRODUCTS; ALONE AND AS A SIDE STREAM

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**Introduction**
The Baltic MUPPETS project is an investment in a blue bioeconomy that aims to deliver economically and environmentally viable businesses with a positive socio-economic impact. The project sets out to create an entirely new value chain based on small blue mussels, enabling a new circular economy in the Baltic Sea region and supporting mussel farmers throughout Europe to develop, diversify and scale their existing businesses. Ultimately, Baltic MUPPETS aims to bend today's linear nutrient economy, by creating a profitable pet-feed market from recycled nutrients.

**Small blue mussel business cases**
Blue mussel production today is wide-spread through Europe and is mainly produced for food. They provide important ecosystem services such as nutrient recycling, habitat provision, and water quality improvement. Small mussels in the Baltic Sea are small by nature due to the low salinity and have had no commercial market but still provide these valuable ecosystem services (Holbach *et al.*, 2020; Vaher *et al.* 2024), and provide an opportunity for sustainable protein production and circular nutrients. Small mussels are also a side stream from traditional commercial food-mussel production, and valorising them provides an opportunity for stronger business models for mussel farmers also in higher salinity areas.

Projects and studies have also shown blue mussel production could be subject for carbon- and or nutrient credit schemes (BalticBlueGrowth, 2019; Lindahl *et al*., 2005; van den Burg, 2022).

**Case business models**
ECOPELAG in Sweden produces small mussels in the Baltic Sea. Their business is based on donations in exchange for delivered ecosystem services. Pet food can support a more sustainable business case. Wittrup Seafood, a commercial mussel producer in Denmark, and Kieler Meeresfarm, small-scale mussel producer in Germany, both look for market opportunities for unutilised "waste mussels".

**Products**
Poultry feed can be produced from mussels (Jönsson, 2009) including small mussels. The shells provide important calcium carbonate, and the meat contains the needed Omega-3 and amino acids (Jönsson, 2009). The project Five Tons of Green Fish by SLU showed decrease in greenhouse gas emissions by 70% of mussel meal compared to conventional fish feed.

Pet food is a higher value alternative than feed, but requires more refining in the meat and shell separation. In Baltic MUPPETS, Östersjöfabriken built up a processing facility with adapted machinery to process the smaller mussels. This resulted in the product Sjötugg, a sustainable pet snack from 100% mussel meat. The product provides a low-fat, high protein and high Omega-3 alternative, especially suitable for dogs with food sensitivities due to the novel protein source leading to low allergy or over-sensitivity risk.

The shells left over from feed production can also be valorised. Grobruket has developed a soil enhancer and fertilizer; Havsmineral and Havsnäring ("Sea Mineral and "Sea Nutrients") based on mussels and fish side-streams, showing good nutritional properties for plant germination and growth support. The mussel shells provide good liming properties due to the high calcium carbonate content.

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# THE LASTING EFFECTS OF EMBRYONIC TEMPERATURE ON ATLANTIC SALMON IMMUNITY TO *Yersinia ruckeri*

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**Introduction**

In both Scottish and Norwegian aquaculture, around one fifth of Atlantic salmon (*Salmo salar*) are lost during the seawater phase of production due to a wide range of pathogens, underscoring the need for more robust and resilient fish. One possible contributor to low robustness is suboptimal temperature during embryogenesis. In many species, including Atlantic salmon, exposure to environmental stimuli such as temperature during embryogenesis can induce long-lasting effects on physiology and metabolism, a phenomenon known as metabolic programming (Ziqiang et al., 2019). Notably, embryonic temperature in salmon is positively associated with growth rate during early development (Gorodilov, 1996).

In the COOLFISH project, we hypothesised that relatively higher embryonic temperature regimes sometimes used in salmon aquaculture may lead to biased resource allocation towards growth, with negative effects on immune system development. In support of this hypothesis, a recent study demonstrated embryonic temperature-dependent differences in Atlantic salmon mortality following *Y. ruckeri* exposure at the alevin stage, indicative of higher robustness at 4 °C than 8 °C (Malik et al., 2025). Currently, it remains unclear whether the impact of embryonic temperature on fish robustness is maintained until later life stages due to long-term changes in immune function. In this study, we investigate how changing temperature solely from fertilisation to the eyed stage of Atlantic salmon embryogenesis influences the functional immune response to *Yersinia ruckeri* infection using head kidney (i.e., primary immune tissue) samples at the parr stage using a transcriptomic approach.

**Methods**

Atlantic salmon embryos were reared under three temperature regimes (4 °C, 6 °C, and 8 °C) from fertilisation to the eyed stage, after which all groups were maintained at 8 °C until start-feeding, followed by 10 °C until sampling (Figure 1A). Approximately nine months after the end of the embryonic temperature treatment, head kidney tissue was sampled from 120 parr-stage salmon ($n$ = 4 per treatment group, for both control and *Yersinia ruckeri*-infected fish) across five timepoints (T): T1 (24 h), T2 (48 h), T3 (72 h), T4 (1 week), and T5 (2 weeks) post-infection (Figure 1A). Total RNA was extracted using a Promega Maxwell instrument before its purity and integrity was confirmed. mRNA-seq libraries were produced and sequenced on an Illumina platform by Novogene. Alignment and quantification of reads was carried out through the NF-core RNA-seq pipeline (v3.14) using STAR and RSEM respectively. Using edgeR (v4.4.2) a generalised linear model was constructed to assess differences in infection dynamics across temperature groups, track the immune response over time, and explore interactions between these factors. Additionally, self-organising map (SOM) clustering was applied to identify key gene clusters responsive to infection and temperature. Differential expression tests were conducted for all pairwise temperature comparisons (4 °C vs. 6 °C, 4 °C vs. 8 °C, and 6 °C vs. 8 °C), across all timepoints, with Gene Ontology (GO) enrichment analysis used to interpret the biological functions of significantly differentially expressed genes.

**Results and Discussion**

Pairwise differential gene expression analysis of head kidney control samples (n=20 per temperature group, drawn from T1-T5 control samples) revealed 2,067 genes to be constitutively differentially expressed between temperature groups ($p$-adj < 0.05, logFC>0.5) (Fig. 1.B). The 8 °C group was a clear outlier, showing a much higher number of differentially expressed genes compared to both the 6 °C (466 genes) and 4 °C (2033 genes) groups. In contrast, the 4 °C and 6 °C groups were more similar, with only 60 differentially expressed genes. Cumulative mortality over the course of the *Yersinia ruckeri* challenge differed by embryonic temperature group, with the 4 °C group showing the lowest mortality (~22%), followed by the 6 °C (~31%) and 8 °C (~35%) groups. 3,935 genes were differentially expressed in the head kidney in response to *Yersinia ruckeri* infection across

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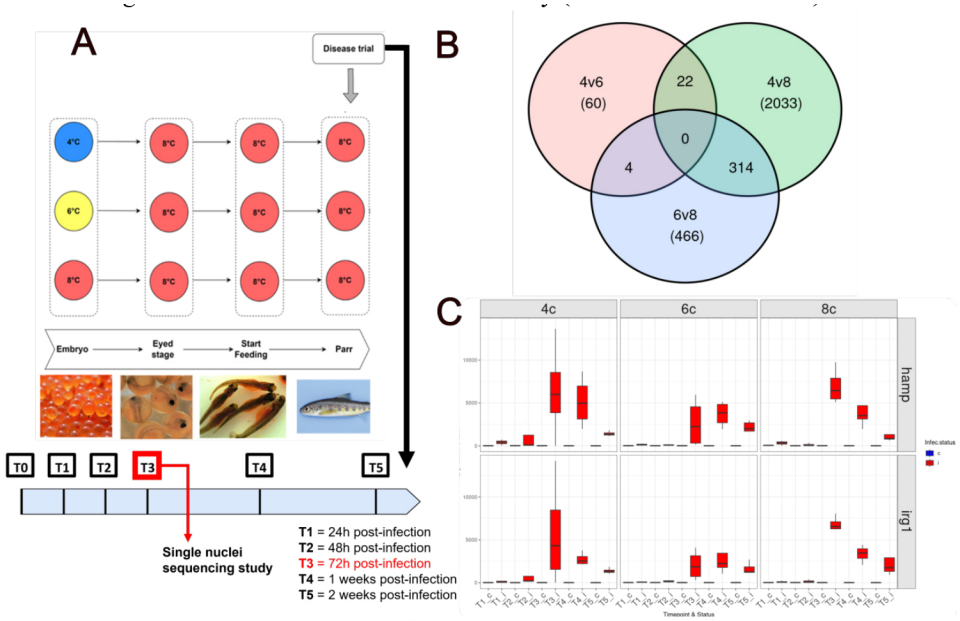


**Figure 1: (A)** Experimental design. (**B**) Venn diagram of differentially expressed genes between temperature groups for n=20 control samples each. (**C**) Boxplots of candidate immune genes showing response kinetics across the infection timecourse.

all timepoints and temperature groups (*p*-adj <0.01, logFC >1). Of these, 2,475 genes displayed temperature group specific expression patterns. Temporal profiling of key immune marker genes revealed T3 to be when the peak inflammatory response occurred in all temperature groups (Figure 1C). However earlier upregulation of numerous immune genes was observed in the 4 °C group, suggesting a more rapid immune activation. This pattern was further supported by enrichment of immune-related GO terms specific to early timepoints (T1 and T2) in the 4 °C group.

Biological interpretation of differential gene expression among temperature groups is ongoing. However, our findings provide clear initial evidence that embryonic temperature exerts long-lasting effects on head kidney gene expression, persisting to the parr stage and shaping the host immune response to bacterial infection. We have also generated head kidney single-nucleus RNA-seq data from timepoint three to gain insights into the cell-type specific nature of these temperature-induced effects during the peak inflammatory response.

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# PROFILE OF LIPIDS AND FATTY ACIDS IN FIVE MUSCLES OF WILD AND FARMED BLUEFIN TUNA (*Thunnus thynnus*)

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## Introduction

Fatty acid composition and lipid contents are a key nutritional and ecological indicator in fish with higher metabolism and widely migratory fish, such as bluefin tuna (*Thunnus thynnus*). The distribution of these lipids is not homogeneous in all the muscles (Nakamura *et al.* 2007). In this study, we assess the quality of Atlantic bluefin tuna muscle by analysing five anatomically distinct muscle tissues, selected to capture potential variability in lipid content and fatty acid composition across the body. Assessing the biochemical composition of these cultured individuals is essential to determine the quality of the product being obtained and to guide future improvements in farming practices.

## Material and methods

Cultured juveniles came from fertilized eggs obtained from commercial tuna floating cages belonging to Ricardo Fuentes Group and placed in San Pedro del Pinatar (Spain) in mid-July 2020. Eggs were hatched and cultured in ICTS-ICAR (Infrastructure for Atlantic Bluefin Tuna Aquaculture, www.icar.ieo.es) facilities, moved to ICRA (Infrastructure for Controlling Bluefin Tuna Reproduction) when became fingerlings, and fed on dry pellets until they reached an average weight of ~100 g. Then diet was change to bait consisting in sardinella (*Sardinella aurita*), European anchovy (*Engraulis encrasicolus*), and Atlantic mackerel (*Scomber colias*). Cultured fish included in the study were individuals that died accidentally due to collisions between November and December, with a mean body weight of 431.1± 170.5 g. Wild specimens were captured in the Bay of Mazarron (SE Spain) by trolling in late September, with a mean body weight of 422.4±70.5 g. Samples were collected from neck muscle (NM), anterior dorsal muscle (ADM), anterior ventral muscle (AVM), posterior dorsal muscle (PDM) and posterior ventral muscle (PVM), and analized to determine lipid content and fatty acids. The data were arc-sin transformed before Kruskal-Wallis tests followed by Bonferroni-corrected post hoc comparisons. Correlation between body weight and lipid components was assessed using Spearman's rank correlation.

## Results

There is a significant positive relationship between weight and lipid percentage in farmed animals ($P = 0.007$), especially in certain muscles such as NM (Table 1). In wild animals, no significant relationship was found between weight and lipid percentage ($P = 0.283$). The percentage of lipids is significantly higher in cultured juveniles in all muscles, especially significant in AVMs (13.5%). The total MUFA is significantly higher in the culture group especially due to the high oleic acid content (20.3%). Both n-3 PUFAs and total PUFAs are significantly higher in wild tunas in the five muscles analyzed, as well as the percentage of DHA. Within the cultured group, the highest PUFA values are found in the two dorsal muscles, although without significant differences. In wild animals, ADM is the one with the highest percentage of PUFAs, with significant differences in most of these fatty acids between the different muscles. The n-3/n-6 and DHA/EPA ratio was significantly lower in farmed fish ($P < 0.001$).

## Conclusions

There is a significant positive relationship between weight and lipid percentage in farmed animals in contrast to wild animals. This pattern could be influenced by factors such as the differentiated metabolic activity between farmed and wild animals. Full-cycle tuna culture produces fish with higher fat content than wild tuna, especially in the ventral muscles, but with a lower percentage of PUFAs in all muscles. Similar results were observed in studies of *Thunnus thynnus* (Miras *et al*. 2024) and *Thunnus orientalis* (Nakamura *et al.* 2007), indicating that the fat composition of the feed was not directly reflected in the muscles of farmed tuna, suggesting proper lipid metabolism. As *Nakamura et al.* (2007) suggested the composition of fatty acids may not depend directly on the food, as factors such as lack of physical exercise or excess energy could be an explanation and further studies should be conducted.

*(Continued on next page)*

| | Cultured | | | | |
|---|---|---|---|---|---|
| | **NM** | **ADM** | **PDM** | **AVM** | **PVM** |
| **Lipids** | 7.4 ± 3.6 | 4.4 ± 1.3 | 4.1 ± 0.6 | 13.5 ± 9.7 | 4.2 ± 1.1 |
| **Total MUFA** | 29.2 ± 9.7 | 24.1 ± 6.0 | 23.4 ± 6.7 | 30.8 ± 9.1 | 25.6 ± 8.8 |
| **Total n-3 PUFA** | 31.8 ± 12.2 | 38.1 ± 8.2 | 38.4 ± 6.5 | 25.3 ± 16.0 | 34.7 ± 13.2 |
| **Total PUFA** | 37.2 ± 14.0 | 44.1 ± 9.7 | 44.5 ± 8.0 | 30.1 ± 18.8 | 40.7 ± 14.5 |

| | Wild | | | | |
|---|---|---|---|---|---|
| | **NM** | **ADM** | **PDM** | **AVM** | **PVM** |
| **Lipids** | 3.9 ± 0.3 | 3.0 ± 0.5 | 3.0 ± 0.5 | 4.0 ± 1.1 | 4.2 ± 1.7 |
| **Total MUFA** | 13.3 ± 1.6 | 13.3 ± 1.6 | 13.4 ± 1.1 | 18.8 ± 4.4 | 13.3 ± 0.8 |
| **Total n-3 PUFA** | 47.3 ± 3.5 | 50.5 ± 4.3 | 47.8 ± 1.4 | 40.7 ± 5.9 | 48.2 ± 1.2 |
| **Total PUFA** | 52.4 ± 3.7 | 56.0 ± 4.6 | 53.4 ± 1.4 | 45.6 ± 6.3 | 54.0 ± 1.1 |

**Table 1**. Percentage of lipids and total fatty acids in the different muscles of the two groups (average ± std).

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## Acknowledgements

To the staff of ICTS-ICAR (IEO, CSIC) and to the Ricardo Fuentes Group company. This work has been funded by the NEWSPEC-MURCIA project, funded by EMFAF (European Maritime, Fisheries and Aquaculture Fund).

# EFFECT OF PHOTOPERIOD ON BLUEFIN TUNA (*Thunnus thynnus*) LARVAL REARING

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## Introduction

In the production of juvenile bluefin tuna, larval rearing is one of the critical stages. Several studies have been carried out to improve growth and survival at this stage. Photoperiod is one of the most important conditions affecting survival and growth in larval rearing. In the case of bluefin tuna this factor is still more important. In a previous study, Ortega *et al.,* (2014) observed that it seems that long photoperiod lead to a greater mortality and short photoperiod led to a smaller growth. This study tries to increase the knowledge of the effect of the photoperiod on bluefin tuna larval rearing.

## Materials and methods

Fertilized bluefin tuna eggs, coming from broodstock in floating net cages owned by Ricardo Fuentes e Hijos, off the Cartagena coast (El Gorguel SE Spain) were transported to the Spanish Institute of Oceanography (IEO) Planta Experimental de Cultivos Marinos (Puerto de Mazarrón, Murcia, Spain). After cleaning and counting the just collected eggs (145,000 buoyant eggs) were separated and directly inoculated in larval rearing tanks. Initial density was 10 eggs/l. The trial was carried out in 12 1200 l cylindrical tanks in four replicates. Three different photoperiod conditions (12hL:12hD, 16hL:8hD, 24hL:0hD) were assayed. The light intensity was about 800 luxes and temperature ranged between 22.0 and 23.5ºC. The renewal rate was 100 %/day of filtered sea water (37ppt salinity). Tanks had water and air bottom inlet, and water outlet from surface. Surface cleaners were used from 3dph. Aeration was increased during the night in order to avoid sinking of the larvae. Feeding during all the period was rotifer enriched with Algamac 3050 (Bio-Marine Inc) and Origreen (Skretting) completed to 10 rot/ml. every day during photophase, but trying to eliminate the unenriched rotifers during the night time. Phytoplankton (Tiso) was also added to the tank twice per day. A sample of 20 larvae were measured at 3, 6, 10 14 and 17dph. At the end of the experiment (17dph) all the survival larvae were counted.

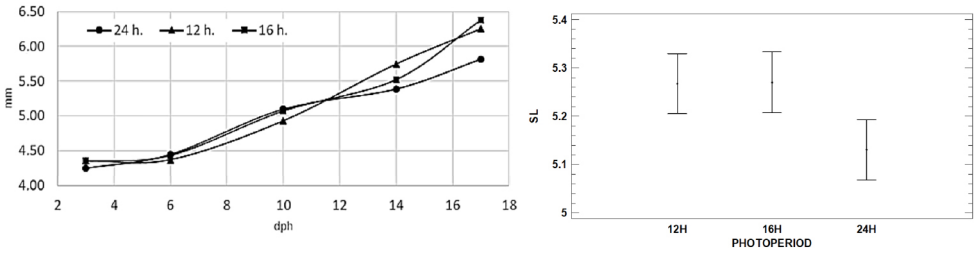


Figure 1. Effect of photoperiod on bluefin tuna larvae growth; left: larval growth (SL mm) in different photoperiods; right: ANOVA between SL and different photoperiods with Least Significant Difference (LSD) intervals, for all the experiment.

| Photoperiod | Survival rate% |
|---|---|
| 12hL:12hD | 7.70±2.30 (a) |
| 16hL: 8hD | 2.68±1.79 |
| 24hL: 0hD | 3.38±2.15 |

*(Continued on next page)*

**Results**

With regard to growth rate, results obtained at 17dph with 12hL and 16hL were similar and significant better ($p<00.1$) that those obtained with 24hL photoperiod (Figure 1).

Results on survival (%±SD) in photoperiod experiment are summarized in the table. In order to normalize the data, survival rate have been transformed according to arch sin function. Letter points significant differences ($p<00.1$)

**Discussion and conclusion**

Results of this study show that 24hL lead to a low survival rate and smaller growth. These results are partially in line with those obtained previously by Ortega *el al.,* (2014) in which 24hL lead to a low survival rate but a greatest growth. In a similar study, Kumon *el al.*, (2018) noted that survival of Pacific bluefin tuna larvae (*Thunnus orientalis*) under 24hL was significantly higher than 14hL, while there was no significant difference in growth between different used photoperiods (14hL and 24hL). In a similar work but on *Thunnus albacares* larvae, Partridge *et al.,* (2011) found that larvae cultured under the 24hL were significantly larger and exhibited more advanced development than those reared under the 12hL photoperiod, suggesting that the improved survival and growth achieved under a continuous photoperiod is due to the extended foraging time combined with the prevention of mortality caused by night-time sinking. The reasons for these different results are unclear. In the previous study with this species, the low survival rate of fast-growing larvae could be related to size-dependent mortality. A short photoperiod leads to slower growth, and the larvae did not experience the second mortality peak, related to notochord flexion and the onset of metamorphosis. However, extending the rotifer feeding period from 13 to 17dph, as occurred in this trial, could induce selective mortality in the larger larvae exposed to long photoperiod, which could partially explain the results obtained in the present experiments. Therefore, further studies are needed to clarify this contradiction and determine the effect of photoperiod on the growth and survival of bluefin tuna larvae.

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# ECOTOXICOLOGICAL EFFECTS OF PESTICIDES, ANTIMICROBIALS, AND THEIR MIXTURES ON DUCKWEED *Lemna Minor*

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## Introduction

Anthropogenic chemical pollution has the potential to pose one of the largest environmental threats to humanity, but global understanding of the issue remains fragmented. Water pollution becomes alarming since anthropogenic activity results in the contamination of water bodies with various contaminants, which in turn reduces ecosystem functioning and poses human health risks. Pesticides and pharmaceutical and personal care products (PPCPs) stand out for their widespread occurrence and highly diverse biological effects and are considered as emerging contaminants (ECs). Pesticides can enter adjacent water bodies through different ways and their residues may be overtaken by non-target organisms, leading to chronic lethal and sublethal effects. In the European Union, herbicide terbuthylazine (TBA), a member of the chloro-s-triazine group, has emerged as one of the most frequently detected herbicides in water bodies. Triclosan (TCS), is one of the most widely used antimicrobial agents in both pharmaceuticals and personal care products, and it is a frequently detected emerging organic contaminant in the environment. Despite frequent detection in surface waters, the mixture effects of these emerging contaminants on the aquatic organisms remain unclear. To address the knowledge gap of the effects of emerging contaminants and their mixtures, we aimed to test mixed exposures of frequently detected pesticide Terbuthylazine (TBA) and antimicrobial Triclosan (TCS) to duckweed *Lemna minor*.

## Methods and materials

Experiments with *L. minor* were conducted in the controlled environment growth chambers. Duckweeds were exposed to 6 different Triclosan (TCS) and Terbuthylazine (TBA) concentrations (range from 1 to 200 $\mu$g $L^{-1}$). TCS and TBA were exposed to *L.minor* in glass jars (450 ml), at 5 replicates in a 200 ml Steinberg growth medium (pH 5.5 ± 0.20). Single TCS and TBA as well as their mixture (TCS+TBA) toxicity experiments following 10 days exposure were conducted according to standardized OECD protocol No. 221 (OECD 2006). Mixture of TCS and TBA were formed based on effective concentration ($EC_{50}$) values from single chemical toxicity, where selected TU (toxic units) (0.25 – 2 TU) were used in a mixture. After 10 days of exposure *L.minor* growth parameters (total frond area (FA) and oxidative stress parameters (lipid peroxidation (MDA)) were evaluated.

## Results

Results showed that single exposure to triclosan (TCS) and terbuthylazine (TBA) inhibited *L. minor* growth. Strong negative correlation was detected ($r = -0.73 – -0.84$, $p < 0.05$) between concentration of both tested chemicals and *L. minor* frond area. Triclosan was a little bit more toxic to duckweed growth as effective concentration of TCS was slightly lower in comparison to TBA (observed $EC_{50}$ were 34.99 $\mu$g $L^{-1}$ and 39.11 $\mu$g $L^{-1}$, respectively). Similalrly to a single exposure of TCS and TBA, it was discovered that with increasing contaminant concentration of mixture (toxic unit, TU), the growth of *L. minor* had tendency to decrease ($r = -0.92$, $p < 0.05$). Observed $EC_{50}$ of a mixture (TBA+TCS) was 37.34 $\mu$g $L^{-1}$. In our experiment it was found that mixture of chemicals under was sligltly antagosnistic as predicted $EC_{50}$ of mixture was predicted to be 36.98 $\mu$g $L^{-1}$, and observed was slightly higher (37.34 $\mu$g $L^{-1}$).

## Conclusions

Duckweed Lemna minor growth was inhibited by single and combined exposure to triclosan and terbuthylazine. The toxicity of chemicals in the mixture did not differ significantly, although it was slightly antagonistic.

# OPTIMIZING STOCKING DENSITY AND SEMI-MOVABLE PADDLEWHEEL AERATION FOR ENHANCED PERFORMANCE AND PROFITABILITY 0F NILE TILAPIA (*Oreochromis niloticus*) IN CONCRETE POND CULTURE

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This study examined the effects of stocking density on water quality, growth performance, and economic viability in Nile tilapia (*Oreochromis niloticus*) cultured in concrete ponds aerated using a semi-movable paddlewheel aeration system. Three stocking densities (80, 120, and 160 fish/$m^3$), representing the experimental groups were evaluated over a 90-day production cycle. Results confirmed that while higher densities significantly increased total biomass, they adversely affected dissolved oxygen levels, ammonia accumulation, survival, and growth performance. In particular, the highest density group (160 fish/$m^3$) exhibited elevated unionized ammonia concentrations, lower survival, and reduced final body weight, despite generating the highest gross profit. Conversely, the 120 fish/$m^3$ treatment achieved an optimal balance between growth performance, size uniformity, survival, and economic return, with a benefit–cost ratio. Increased density was associated with impaired water quality, reduced survival rates, and a shift toward smaller size classes. Although the semi-movable paddlewheel system improved oxygen distribution and waste dispersion, its capacity was limited under high-density conditions. These findings underscore the importance of aligning stocking density with aeration design and support the use of moderate density (120 fish/$m^3$) and adaptive aeration as critical components for achieving sustainable intensification in concrete-based tilapia production systems.

# COMPARISON OF WATER SOURCE EFFECTS IN AQUAPONICS: TAP WATER AND RECLAIMED WATER FROM URBAN WASTEWATER TREATMENT PLANT

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**Introduction**

Aquaponics is a non-traditional approach to producing both fish and crops, integrating aquaculture with hydroponic systems. It can be implemented in areas with rocky, saline, or non-arable soils, without the need for fertilizers or chemical pesticides. This method helps to reduce land use and degradation, eases pressure on water supplies, and lowers the environmental footprint of agriculture [1].

Urban wastewater is increasingly being utilized in agriculture due to growing water scarcity and stress, alongside rising populations, greater volumes of wastewater, and pollution incidents. Urban environments should explore new opportunities for repurposing wastewater, channeling more of it inland for productive uses—not just in agriculture, but also in aquaculture and aquaponics [2,3]. For instance, reclaimed water can lessen aquaculture's dependence on freshwater sources, enabling it to be located near urban wastewater treatment facilities. This would reduce production costs and provide communities with fresh, protein-rich food [4]. However, limited public acceptance and the absence of clear regulations remain major challenges [5].

The main objective of this work is to demonstrate (i) the efficiency of the advance tertiary wastewater treatment to produce acceptable water quality for aquaponics reuse; and (i) thatthe yields of plant and fish in tap water-fed aquaponic systems are comparable to those obtained with reclaimed water from urban wastewater treatment plants. Within the framework of the project Aquaponics from WAstewater REclamation (AWARE) project, GA No. 101084245, a small-scale experimental aquaponic system has been integrated with a pilot urban wastewater treatment facility at URJC in Madrid, Spain. Wastewater undergoes advanced tertiary treatment specifically adapted to feed the aquaponic system, which is used to grow lettuce and tilapia. The water quality of the aquaponic farm using reclaimed urban wastewater has been assessed.

Table 1. Total aerobic bacteria and total coliforms (CFU/ 100 mL) present in different water sources within the aquaponic system.

| | **Log C (CFU/100 Ml)** | | | |
|---|---|---|---|---|
| | **Total aerobic bacteria** | | **Total Coliforms** | |
| | **Tap water cycle** | **Reclaimed Wastewater cycle** | **Tap water cycle** | **Reclaimed Wastewater cycle** |
| **Week 1** | 5.60 | 5.67 | 5.36 | 1.27 |
| **Week 2** | 4.03 | 5.65 | 3.90 | 1.39 |
| **Week 3** | 3.28 | 5.10 | 3.20 | 1.73 |
| **Week 4** | 3.64 | 4.41 | 3.04 | 1.41 |
| **Week 5** | 3.44 | 5.96 | 2.74 | 2.20 |
| **Week 6** | 4.73 | 5.52 | 4.51 | 3.97 |
| **Week 9** | 4.31 | 4.28 | 4.27 | 3.84 |

*(Continued on next page)*

**Materials and Methods**

An advanced tertiary water treatment process was applied to further purify the effluent from a wastewater treatment plant (WWTP). This treatment system included sequential modules of Ultrafiltration (UF), electrooxidation (EO), and UVC-LED irradiation, using a total of 60 UVC-LEDs. The UF membrane, made of $Al_2O_3$, had a pore size of 100 nm and dimensions of 0.23 m² by 1.2 m. In the EO module, the anode was composed of titanium with mixed metal oxides (Ti/MMO), while the cathode was titanium. Operating parameters included a surface area of 270 cm², a current of 5.2 A, voltage between 10–15 V, and energy input of 78.85 J. The UVC-LEDs emitted radiation within a 268–280 nm range, peaking at 275 nm, with a total energy output of 102.99 J. The system operated at a flow rate of 200 L/h.

The study involved a 1 m³ aquaponic system using reclaimed urban wastewater, stocked with 50 Nile tilapia (*Oreochromis niloticus L.*) at a biomass density of 5.7 kg per system, and 72 lettuce plants (*Lactuca sativa*, Batavian Red variety), cultivated across 22 five-week crop cycles. The experiment was carried out over 19 weeks in a small-scale, closed-loop, recirculating aquaculture system (RAS). A control setup using dechlorinated tap water served as the baseline for comparison.

The measurements analyzed involved physico-chemical such as nitrogen and phosphorus species, organic carbon, turbidity, total suspended solids (TSS) apart from temperature, oxygen, electrical conductivity, pH. Microbiological parameters were quantified by serial dilution and plate count method and plate count not only in water, but also in lettuce and fish.

**Results**

Compounds which are toxic to fish in aquaponics are nitrite ($NO_2^-$) and ammonia ($NH_4^+$). During the aquaponic cycle with reclaimed water, levels below the toxicity threshold were achieved. The difference between both cycles was significant in terms of some nutrients such as total phosphorus, reaching over 5 ppm in the reclaimed water source, compared to levels below 0.5 ppm in the tap water source. This is probably because reclaimed water contains more nutrients than dechlorinated tap water. Since nutrients are beneficial to the hydroponic system, this increase may increase the produce yields.

Regarding parameters related to organic matter, similar values have been observed in terms of Chemical Oxygen Demand (COD) (10–40 ppm mg $O_2$/L) and turbidity (<0.5 NTU) in both cycles.

Regarding bacterial load in water within the aquaponic system, total aerobic bacterial growth was slightly higher in the reclaimed water cycle, as this type of water initially contained a certain microbial load. However, levels gradually stabilized over time, reaching values comparable to those observed in the tap water production cycle. This is a highly positive indicator of the water quality within the system. Notably, no significant pathogens such as *Escherichia coli* ,*Salmonella* , *Clostridium perfringens*, MS2 coliphage, Enterococcus were detected in any of the water sources. On the other hand, during the first weeks of the cycle, reclaimed water has fewer coliform bacteria compared to the aquaponic cycle with tap water. However, over the weeks, coliform levels in both cycles become similar.

**Conclusions**

The physicochemical and microbiological parameters analyzed do not indicate that feeding reclaimed water into an aquaponic system affects the yields of plants and fish within the system. Key parameters related to organic matter suggest that organic content in the medium is limited, thus not depleting the available dissolved oxygen and preventing the proliferation of anaerobic bacteria that produce undesirable compounds such as hydrogen sulfide, methane, or ammonia. It has been demonstrated that the introduction of reclaimed water into an aquaponic system does not result in an increase in bacterial load, nor is this reflected in the bacterial populations found in the animal and plant species within the system. No significant pathogenic species, as previously mentioned, were detected. Meanwhile, levels of aerobic bacteria and coliform bacteria were found to be comparable across both water sources.

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# COMPREHENSIVE PROTOCOL TO CONTROL VIBRIOSIS CAUSED BY *V. vulnificus* pv. *piscis*. ASSESSMENT OF FUNCTIONAL DIETS

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## Introduction

Stress in aquaculture animals increases their susceptibility to infections. One of the most concerning pathogens is *Vibrio vulnificus*, a zoonotic aquatic bacterium whose distribution is expanding due to global warming. It causes warm-water vibriosis in fish, with haemorrhagic septicaemia as its most severe form. Its virulence is linked to the pVir plasmid, which harbours genes like *fpcrp* and *ftbp*, essentials for evading the fish's innate immune system and forming a "blood survival kit"[1]. Recent studies demonstrated that farming conditions have driven the evolution of this pathogen. Therefore, the development of sustainable preventive strategies is urgently need. These strategies could act as bottleneck against the horizontal transfer of adaptation genes, thereby limiting the emergence of more resistant strains and contributing to improve environmental, animal, and human health. This study is part of a project whose main goal is to propose a preventive protocol based on two pillars: functional feeding and vaccination (unpublished results). Specifically, this study focuses on developing a phytobiotic-supplemented diet with stimulatory and protective effects for eels (*Anguilla anguilla*) against vibriosis caused by *V. vulnificus* pv. *piscis*.

## Materials and methods

Phytobiotics and functional feeds. The activity of a series of phytobiotics against *V. vulnificus* strains was determined by calculating the minimum bactericidal concentration (MBC). Functional diets (A and B), including most the most active products (0.9 g phytobiotic/kg feed), were prepared. The best candidate (based on their immunostimulatory effect) was used to prepare a more concentrated feed (1.8 g phytobiotic/kg feed).

Feeding trials. In the stage 1, after 3-weeks acclimation period with the control diet, eels were feed control or A or B functional diets for 4 or 5 weeks. In stage 2, eels were fed the control diet during an acclimation period of 3 (trial 1) or 4 weeks (trial 2). Thereafter, each group was subdivided into 3 subgroups and fed daily the control or functional diets selected in stage 1 for five weeks. The feeding rate was 2% in all assays.

Blood sampling. Blood samples were collected before and after 4 and 5 weeks to obtain plasma.

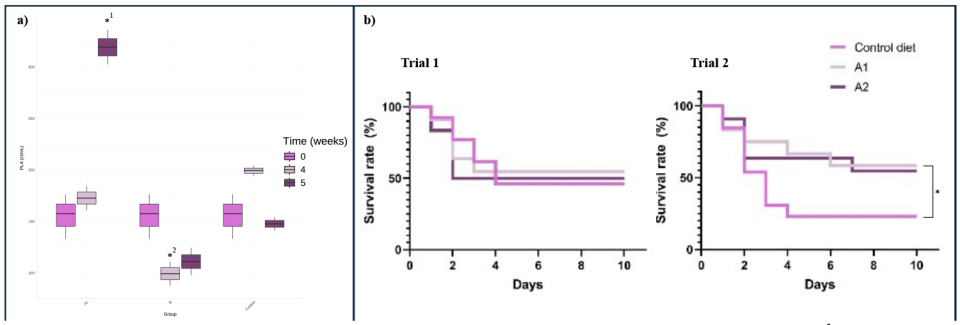


*Figure 1. a) Plasma lysozyme activity of eels fed control or functional diets. *[1]. Significant differences with the other groups and the pre-feeding values. *[2]. Significant differences with the other groups except for the group fed A1 diet for 4 weeks. b) Eels' survival after challenge with Vibrio vulnificus* pv. *piscis. *Significant differences (p-value<0.1).*

*(Continued on next page)*

Plasma lysozyme activity (PLA). This activity was assessed using a microplate turbidimetric assay. A *Micrococcus lysodeikticus* suspension was mixed with plasma samples (1:10), and absorbance at 450 nm was measured at 0 and after 20 min at 28 °C. PLA (U/mL) was calculated from a standard curve using egg white lysozyme.

Challenges. Infection challenges with *V. vulnificus* CECT4999 strain were conducted after the feeding period by prolonged immersion (1 hour), using the previously estimated infection dose. The protective effect conferred was calculated using the Relative Percentage of Survival (RPS).

Statistics. PLA values were statistically analysed using two-way ANOVA tests and Tuckey's post hoc test with Bonferroni correction ($p<0.05$). Eel survivals were statistically analysed using Kaplan-Meier approach and log-rank test as post hoc analyses ($p < 0.1$).

**Results**

All phytobiotics showed high bactericidal activity against *V. vulnificus*, regardless of the strain (113–173 ppm). UV-1 and UV-9 were selected for the formulation of functional diets (A and B) based on their efficacy and cost-related composition. PLA activity significantly increased in the plasma of eels fed A diet for five weeks (319.2 ± 23.6 U/mL). Based on these results, the A diet — and thus the phytobiotic UV-1 — was selected to assess protection against vibriosis at two concentrations (0.9 g/kg feed and 1.8g/kg feed, A1 and A2 diets respectively). In infection trial 1 (3 weeks of acclimation), eels fed functional diets for five weeks showed a slight reduction in mortality compared to controls, with RPS values of 15.58% (A1) and 7% (A2). Conversely, trial 2 (4 weeks of acclimation) showed a much stronger protective effect, with RPS values of 46% (A1) and 41% (A2).

**Discussion and conclusion**

The rise in vibriosis outbreaks in aquaculture has motivated the implementation of effective control protocols as a priority. This work is part of an ambitious project aim at proposing a protocol that not only prevents the transmission of vibriosis caused by *V. vulnificus* but also limits the spread of virulence genes responsible for the resistance to the fish's innate immune system. In this study, we evaluated the best phytobiotic to protect eels. UV-1 emerged as a promising candidate for functional diets, provided the eels are adequately acclimated. Furthermore, its broad-spectrum potential is further supported by previous evidence of its efficacy against *Vibrio. parahaemolyticus* infections in shrimp[2]and at the same time minimizing antibiotics treatments, is the major challenge in shrimp aquaculture. A sustainable strategy could be to include immunostimulants in diet. Phytobiotics, harmless plant extracts with immunostimulatory and biocidal activities, are promising candidates. In this study, we evaluated the effectiveness of two diets (E and F. Based on our results, we propose the following preventive protocol: upon arrival at the farms, eels are acclimated in tanks. Subsequently, vaccination is carried out at the glass eel stage and includes: the vaccination with Vulnivaccine via immersion and boosters with the Fpcrp-Ftbp subunit vaccine (unpublished results) during the fattening period, administered via nanoparticles-enriched feed (a) or immersion (b). Moreover, under stress conditions eels could receive a UV-1-supplemented diet for 5 weeks to enhance their resistance to vibriosis. Routine vaccination resumes once welfare normalises.

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# *IN VITRO* AND *IN VIVO* EFFECTS OF BIOACTIVES FROM TERRESTRIAL AND MARINE BY-PRODUCTS AND LOW VALUE BIOMASS IN GILTHEAD SEABREAM (*Sparus aurata*) POST-LARVAE

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**Introduction**

In 2022, an estimated 1.05 billion tonnes of food were wasted globally, with fruits and vegetables being the most misused types (UNEP, 2024). Therefore, the recovery and valorisation of bioactive substances from those low value biomass and by-products is a challenging and important task. Not only land-based by-products have potential, since marine by-products have evolved over time from being considered as a secondary material to an opportunity for value-added products. Those bioactives have potential for boosting fish mucosal health and endogenous antioxidant capacity, and specifically proactively reinforcing the health status implied in the defence against pathogen and environmental challenges during developmental stages, such as post-larvae stages. To assess the potential of these eco-innovative functional by-products and value-added bioactives as fish health boosters, it is essential to previously characterize and evaluate their potential antioxidant/immunomodulatory activity.

Thus, the objective of this study was to evaluate, through sequential *in vitro* and *in vivo* experiments, the antioxidant and immunomodulatory activities of terrestrial and marine by-product extracts in gilthead seabream (*Sparus aurata*) post-larvae.

**Material and Methods**

Nine extracts from by-products and low value biomass were tested, 4 of terrestrial origin and 5 of marine origin. Those of terrestrial origin were 2 extracts of pomegranate, one rich in punicalagin and the other rich in ellagic acid (PG and EA, respectively), an extract rich in citrus flavonoids (CF) and a grape seed extract (GSE). The marine ones were extracts of *Rhodomonas lens* (RH), *Osmundea pinnatifida* (OS), *Gracilaria sp.* (GR), *Desmodesmus sp.* (DE) and *Dyctiota sp.* (DY). Extracts phenolic compounds and flavonoids content, as well as their antioxidant activity by ABTS and FRAPs activities were assayed. An *in vitro* screening of the extracts antioxidant and immunomodulatory activities was performed using seabream head kidney leukocytes. Specifically, viability, respiratory burst, peroxidase content and phagocytic capacity of the leukocytes were analyzed. Based on the results obtained on the in vitro asssays, two extracts were chosen, one of terrestrial origin and the other of marine origin (PG and RH) to be tested *in vivo* at two dietary dosages in seabream post-larvae. This experiment (CEEA_ULPGC 33-2023) was carried out from 49 days post- hatch (dph) to 90 dph, testing each diet in quadruplicate tanks with 600 larvae/tank. After the feeding period, a stress challenge was performed by exposing 30 larvae from each tank to air for 2 minutes and observing their recovery for 24h. The analyses of this experiment are currently in progress.

**Results**

Regarding the *in vitro* results, different trends were observed depending on the origin of the extracts (i.e. terrestrial or marine). In the case of terrestrial extracts, a higher content of phenolic compounds and flavonoids was observed ($p$-value < 0.0001), which resulted in a higher antioxidant capacity ($p$-value < 0.0001) compared to marine extracts. Among the terrestrials, GSE presented the highest levels in phenolics compounds and flavonoids ($p$-value < 0.0001), which was related to a higher antioxidant capacity according to ABTS ($p$-value < 0.0001) but not by FRAPS, where the best results were obtained with pomegranate extracts ($p$-value = 0.0003). This may be due to the dependence of the antioxidative capacity of the extracts on their structure, presence of hydroxyl groups or their glycosylation (Parcheta et al., 2021) rather than their total content. On the other hand, a more direct relationship between phenolic compounds and flavonoids with the results in ABTS and FRAPs was observed in marine extracts, with the best candidate being RH. All compounds, except RH and OS, presented an inverse relationship between dose and cell viability. In terms of immunomodulatory activity, the marine compounds showed generally higher activity, possibly caused by the presence of Marine Sulphated Polysaccharides (MSP) (Petit et al., 2024).

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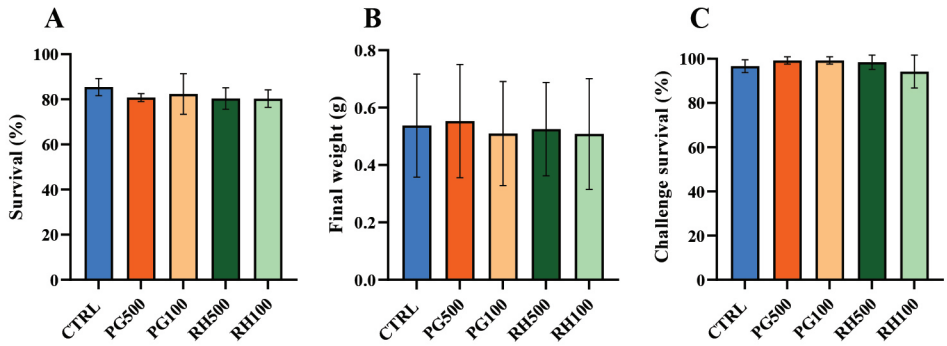


**Figure 1**. Survival rate (**A**), final weight (**B**), and challenge survival rate (**C**) of *S. aurata* post-larvae supplemented with different extracts and inclusion percentages. The results are expressed as the average ± SD. No statistically significant differences were observed from the one-way ANOVA analysis.

Considering the above, PG and RH were chosen as candidates for the *in vivo* assay. The results obtained so far show that supplementation with these extracts did not affect either growth or survival of seabream post-larvae. In addition, survival to a stressor such as air exposure was not affected either, with all groups showing values above 90%.

The rest of the analyses: histology, RNA sequencing and antioxidant enzymes activity, parameters that will provide valuable information, are currently in progress.

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# THE EFFECT OF BOAT NOISE ON BEHAVIOUR AND PHYSIOLOGY OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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**Introduction**

The impact of anthropogenic noise on marine ecosystems has emerged as a critical area of research in recent years. Increased shipping traffic, marine construction, underwater mining, and recreational boating contribute significantly to noise pollution, altering the natural acoustic landscape. Fish are particularly sensitive to these changes, as they rely on sound for communication, navigation, and predator avoidance.

Noise acts as a potential stressor, inducing both physiological responses (increased cortisol secretion, changes in plasma metabolites, and expression of heat shock proteins) and behavioral changes in fish (startle, avoidance, altered foraging, and disrupted collective dynamics) (Wysocky et al., 2006; Cox et al., 2018).

This study adopts a dose-response approach to explore the relationship between sound intensity and both physiological and behavioral variables in European seabass exposed to boat noise, determining how these parameters mirror their respective variations and at which intensity noise may potentially cause harm.

**Materials and Methods**

We exposed 160 European seabass to sound stimuli realistically reproducing fishing boat engine noise. Fish were housed in experimental tanks (350 l) in groups of five, with each tank exposed to one of six distinct sound levels (95-154 dB, re 1 µPa) for one hour.

After exposure, we collected fish plasma following anaesthetic overdose and stored samples at -20°C for cortisol and glucose/lactate analysis via radioimmunoassay (RIA) and an automatic analyser (BIONSEN, EKF Diagnostics). Physiological indicators were compared across treatment Levels through ANOVA.

Behavioral data were collected on videos during three non-consecutive 10-min intervals before exposure and other three 10-min intervals during exposure. A focal animal, continuous sampling method was used to collect data on swimming behaviour (forward swimming, hovering). DeepLabCut, a machine learning video analysis software, was trained to track fish and collect data about swimming speed and distance from other fish. Behavioural data were analysed separately for each Level of exposure via Generalized Estimating Equations, with sampling interval as a fixed factor and fish identity as a random factor.

**Results and Discussion**

The percentage of time spent swimming forward increased significantly with exposure to sound for all Levels between 0 and 3 ($p < 0.001$ for all models) in the first 10 min of exposure, but remained increased for longer with increasing sound intensity, to the point that no return to baseline was observed for exposure to Level 3; a delayed increase in forward swimming was observed in Level 4 ($p < 0.001$) and no increment was observed in Level 5 ($p = 0.644$). Hovering showed a mirroring pattern, decreasing significantly upon exposure to noise ($p < 0.001$), and taking longer to revert to baseline (if at all) with increasing sound intensity up to Level 3. As already observed for swimming, such response was delayed in Level 4 and absent in Level 5. Average swimming speed significantly increased in the first 10 min of exposure compared to all other sampling intervals, in Levels 0 to 3 ($p < 0.05$), while no change was observed in Levels 4 and 5.

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Overall, these results indicate that noise exposure increases swimming activity, in agreement with previous studies (Buscaino et al., 2020). However, the increment in swimming speed was short-lived and independent of sound pressure, representing a transitory response scarcely indicative of the intensity of the effect of noise exposure. Conversely, the increment in forward swimming/decreased hovering and the clear relationship between noise intensity and the duration of such response likely represents the results of stress/arousal due to exposure and of subsequent habituation. The delayed and reduced effect observed in Levels 4 and 5 might reflect a disruptive effect of too intense noise, impeding the expression of an active behavioural response.

Overall, these results indicate that quantification of forward swimming and/or hovering is better suited than swimming speed to measure the impact of noise exposure. These results also suggest that the greatest effects of noise exposure on seabass behaviour occur for noise intensities around or above 130 dB (re 1 μPa).

No differences were found in cortisol, glucose and lactate concentrations across treatments, indicating a lack of conventional physiological stress response despite significant behavioral alterations. This suggests a disconnection between immediate behavioral reactions and typical physiological stress markers in the context of noise exposure requiring further investigation.

**Conclusions**

In conclusion, this study identifies behavioural patterns which seem useful to characterise different aspects of European seabass response to noise exposure. The study indicates 130 dB as the intensity threshold around which seabass can no longer produce adaptive responses. The lack of significant changes in acute stress physiology indicators such as cortisol and glucose levels suggests that current models of stress measurement may require re-evaluation in the context of acute noise exposure. Further research is needed to explore these findings across different environmental contexts and stressors, for instance to assess the long-term impacts of noise pollution on fish survival, foraging, and reproductive success. Understanding the nuanced relationship between stressors and biological responses is vital for formulating effective conservation strategies and ensuring the welfare of marine organisms in increasingly noisy oceans.

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# EFFECTS OF AN ANTIOXIDANT BYPRODUCT AND TAURINE ON MUSCLE CELLS AND EUROPEAN SEA BASS FINGERLINGS FED LOW FISHMEAL DIETS UNDER EXERCISE CONDITIONS

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## Introduction

Mediterranean aquaculture of European sea bass (*Dicentrarchus labrax*) is of high economic relevance, with feed representing the main production cost. The adoption of low fishmeal diets (Oliva-Teles et al., 2015), combined with functional additives (Balbuena-Pecino *et al*., 2022) or management strategies such as sustained swimming, are key approaches to improving the sector's sustainability (García-Pérez *et al*., 2024). In this study, we first evaluated *in vitro* the effects of antioxidant products with potential as additives, including several agricultural byproducts and taurine, on the proliferation and redox status of muscle satellite cells from juvenile European sea bass. Based on *in vitro* results, the most promising additives (antioxidant byproduct (AO-1) and Taurine (T)) were incorporated into experimental low fishmeal diets and tested in a two-month *in vivo* feeding trial with juvenile sea bass, under either voluntary or sustained swimming conditions. The findings provide insights into nutritional and exercise-based strategies to enhance growth and oxidative stress tolerance, supporting the development of more sustainable feeding practices.

## Material and methods

Fingerlings for *in vitro* and *in vivo* experiments (8 ± 3 g) were supplied by Culmarex (Mallorca, Spain). Primary cultures of E. sea bass myocytes were established from epaxial fast muscle. Cells were seeded at $7.5 \times 10^5$ cells/mL in 6 well plates coated with poly-L-lysine and laminin. Cultures were maintained at 23 °C and 2.5% $CO_2$. AOs stock solutions (100×) were prepared under sterile conditions, diluted according to their active compound content, and tested for cytotoxicity using the MTT assay. Products were added on day 3, and cells were incubated with 0.5 mg/mL MTT for 5 h on day 5, for toxicity assessment. Two AOs (AO-1 and AO-2) and taurine (TAU, 10 mM) were selected for the antioxidant capacity assay, which was performed in 96 well plates using $H_2$DCFDA dye (10 $\mu$M) after product incubation, oxidative challenge with TBHP, and fluorescence reading. Quercetin (QC, 10 $\mu$M) was used as positive control. The best AO (AO-1) was tested at two different concentrations (10 ppm and 2 ppm) for RNA analysis. The other treatments, quercetin and taurine, were prepared at the same concentrations as described above. RNA was extracted on day 5 after 24 h of treatment, gene expression related to the Gh/Igfs axis, myogenesis, and antioxidant related enzymes was assessed via RT-qPCR. The *in vivo* trial involved nearly 2,000 tagged fingerlings (5.49 ± 0.03 g) distributed for each condition into three tanks at density of 2 g/L. Conditions differed according to swimming activity: voluntary swimming (VS) or sustained swimming (SS, 1.5 BL/s). Four diets were tested over two months: high fishmeal (D1), low fishmeal (D2), low fishmeal + AO-1 (D3), and low fishmeal + taurine (D4). Fish were fed three times daily at 4% ration of body weight (BW), with ration adjusted to 2.5 % BW after weight control at midpoint. Fish were weighed and measured at the start, midpoint (week 4), and the end (week 8). Biometric parameters (SGR, VSI, MFI, HSI, MSI) were calculated. Statistical analyses (SPSS v30, GraphPad v8) included normality testing (Shapiro–Wilk), homoscedasticity (Levene's test), one- or two-way ANOVA (for *in vitro* or *in vivo* analyses, respectively) with Tukey's or Dunnett's T3 post hoc tests; $p < 0.05$ was considered significant. Biometric parameters (length, weight, and condition factor) were analyzed with a mixed linear model including tank effect, which was minimal.

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**Results and discussion**

*In vitro*, toxicity analyses showed no significant effects at the treatment doses tested. AO-1 displayed the highest antioxidant capacity among products, with effects comparable to quercetin (QC) under oxidative challenge with TBHP, whereas taurine (TAU) and AO-2 did not reduce ROS levels. Based on these results, AO-1 and TAU were selected for *in vitro* further analyses and as the dietary additive in D3 and D4, respectively, for the *in vivo* trial. RNA analyses under basal redox condition (without oxidative stressors) revealed downregulation of *gshr* in AO-1 at 2 ppm and in QC, accompanied by a trend towards *gpx* upregulation in QC. QC also induced significant upregulation of *igf-2*, *pcna*, and *myod2*, while *igfbp-5b* was downregulated. TAU did not cause significant changes in late-proliferative phase (day 5) myocytes but showed a tendency to upregulate *gshr* and *myod2*, which could indicate potential changes during the differentiation phase (day 8-13).

*In vivo*, final body weight was highest in D1-SS and D4-SS (21.5 ± 0.3 g and 20.9 ± 0.3 g, respectively), both significantly greater than other treatments, whereas D3-SS had the lowest value (19.1 ± 0.3 g). Final SGR was significantly higher in D1, followed by D4 and D3-VS. Additionally, D4-SS exhibited a higher muscle somatic index and a tendency towards a lower mesenteric fat index, indicating better overall performance in this group.

In summary, further analyses are required for the *in vivo* experiment, whereas *in vitro* data indicate that AO-1 may exhibit a redox profile similar to that of QC and could be a promising additive to mitigate stress in E. sea bass fingerlings. However, when included in a more sustainable diet with reduced fishmeal content, it does not fully compensate for nutrient deficiencies needed to optimise growth performance, whereas TAU could provide this compensation and potentially confer antioxidant effects.

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# COMPARATIVE IN VITRO CHARACTERIZATION OF PROTEIN HYDROLYSATES FROM CIRCULAR ANIMAL BY-PRODUCTS

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**Introduction**

Protein hydrolysates have gained significant attention in recent years for their ability to deliver high-quality peptides with nutritional and health-promoting benefits in both human and animal nutrition. Among their bioactive properties, their antioxidant potential is particularly noteworthy, along with their potential to modulate microbial growth and anti-inflammatory effects, making them versatile functional ingredients. Beyond their nutritional value and bioactive properties, protein hydrolysates may offer a sustainable solution for waste valorization, particularly within the framework of a circular economy. A deeper understanding of protein hydrolysates and their functional properties is crucial for optimizing their applications in animal nutrition. This study aimed to evaluate the functional and bioactive properties of five protein hydrolysates derived from shark, fish, insect, and swine by-products, using CPSP90 as reference.

**Materials and Methods**

Four protein hydrolysates (PHs) were produced from different animal by-products: shark cartilage and muscle (SHARK), fish trimmings (FISH), swine meat and bone (SWINE), and whole insect larvae of *Hermetia illucens* (INSECT). Raw materials were hydrolyzed using either enzymatic or chemical-free thermal processing methods, optimized according to raw material type and target peptide profile. Hydrolysates were subsequently spray-dried and stored under dry conditions before analysis. Each hydrolysate was characterized in terms of dry matter, crude protein, lipids, ash, phenolic content, chitin, amino acid profile and molecular weight distribution of peptides. Protein solubility was assessed at pH 3.0, 5.0, and 7.0 using the Bicinchoninic Acid (BCA) method. Antioxidant capacity was assessed based on 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities and Oxygen Radical Absorbance capacity (ORAC). Antibacterial activity was assessed for Eight bacterial pathogens relevant to aquaculture. Bacteria were cultured in 96-well microplates with hydrolysate solutions (40 mg/mL) added to the medium. Optical density (OD600) was measured over time to monitor growth dynamics over a period of 24h. All assays included CPSP90, a commercial soluble fish protein concentrate, with wide application in aquafeeds, as a reference.

**Results**

All PHs showed high protein content (55.1–89.5% DM), with SHARK and SWINE richest in collagenic amino acids (e.g., hydroxyproline), and FISH and CPSP90 showing highest essential amino acid (EAA) proportions. INSECT, though lowest in protein (55.1% DM), was high in aromatic and branched-chain amino acids (BCAAs), with total phenolics representing 2.7% DM.

Molecular weight analysis revealed that FISH and INSECT had higher proportions of small peptides (<1 kDa), while SHARK was rich in small to medium-sized peptides (1–3 kDa). In contrast, CPSP90 and SWINE exhibited a more uniform distribution of peptides across different molecular weight ranges.

INSECT displayed the highest solubility (61–65%) across all pH levels, significantly higher than CPSP90. On the other hand, SHARK showed poor solubility (26–36%), likely due to its hydrophobic collagenic composition.

In terms of bioactive potential, INSECT and FISH exhibited the strongest antioxidant activity in both ABTS (11.8 and 8.9 Trolox eq %DM, respectively) and ORAC assays. SHARK showed the lowest antioxidant potential, aligning with its low content in aromatic and sulfur-containing amino acids. No DPPH activity was detected in any PH.

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Regarding bacterial growth modulation, SWINE hydrolysate consistently inhibited the growth of several pathogenic strains, including *Vibrio parahaemolyticus, V. anguillarum,* and *Aeromonas salmonicida.* In contrast, FISH and SHARK hydrolysates often promoted bacterial growth, particularly of *V. parahaemolyticus, V. anguillarum*, and *Photobacterium damselae* subsp *damselae.* INSECT hydrolysate had mixed effects—enhancing the growth of *V. anguillarum, Pseudomonas aeruginosa,* but inhibiting *Tenacibaculum maritimum*, A. *salmonicida* and *V. harveyi*

**Discussion and Conclusion**

The functional and nutritional profiles of animal-derived PHs varied widely with source and hydrolysis process. FISH and CPSP90 exhibited the highest essential amino acid content, while SHARK and SWINE hydrolysates were rich in collagenic amino acids. The higher proportion of small and small to medium peptides in INSECT, FISH, and SHARK hydrolysates is likely due to enzymatic hydrolysis using alcalase. In contrast, the more uniform peptide distribution in SWINE may be due to the thermal-pressure process of hydrolysis without added chemicals.

INSECT and FISH hydrolysates demonstrated superior antioxidant activity, while SWINE stood out for its selective antibacterial properties. While INSECT also exhibited the highest protein solubility, its effects on bacterial growth were mixed—it enhanced the proliferation of certain bacterial strains. Similarly, FISH and SHARK hydrolysates promoted the growth of specific bacteria, likely due to their high content of small and small to medium peptides, which may support microbial proliferation. While this could raise concerns in some contexts, it also suggests potential for these hydrolysates to be used as components in microbial culture media, as readily available sources of nitrogen and carbon. The selective growth-promoting properties of some hydrolysates could also be harnessed to cultivate beneficial bacterial strains or modulate microbial communities in aquaculture systems.

Overall, these findings support the targeted application of PHs in sustainable feed and food systems and highlight their potential to replace conventional protein sources, contributing to circular bioeconomy goals. Future research should focus on in vivo validation and detailed peptide characterization to fully unlock their functional potential.

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# EARLY DRY FEED INTRODUCTION IMPROVES GROWTH PERFORMANCE OF ATLANTIC COD *Gadus morhua* LARVAE

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## Introduction

One of the major bottlenecks in Atlantic cod (*Gadus morhua*) farming is the consistent production of quality larvae and juveniles. Moreover, nutrition plays a key role in the overall health of larvae. Positive development and optimization of weaning feeding protocols, with alternative live prey and novel micro diets in a co-feeding regime, are essential for enhancing larval survival and growth performance. Early introduction of formulated feeds during the weaning stage, gradually replacing live prey, is an efficient way to reduce overall production costs and minimize external sources of variability, leading to higher and constant production of juveniles. This study aimed to investigate the effects of early dry feed introduction in a co-feeding strategy on growth performance and survival.

## Material and Methods

Atlantic cod larvae (*Gadus morhua*) from Ode AS (Stadsbygd, Norway) were randomly disturbed in triplicate 400 L tanks at 2 days post-hatching (DPH) and kept to 74 DPH. The light regime was 24L:0D, and water temperature ranged from 7.9 to 9.9 ºC. In addition, algae paste (*Nanochloropsis gaditana*, AlgaSpring, The Netherlands) was used throughout the trial (2 to 43 DPH). The live feed was the same for the two treatments, a sequence of enriched rotifers (Ode AS) at 3 DPH, followed by small barnacle nauplii (Cryo-S, 10 DPH), and large barnacle nauplii (Cryo-L, 18 DPH), provided by Planktonic (Trondheim, Norway). Barnacle nauplii were cryopreserved, thawed, and reactivated with saltwater in a separate container, and all live feed was supplied to the tanks through different peristaltic pumps. Regarding dry feed, a microdiet tailored for cod was supplied by SPAROS (Olhão, Portugal), and it was used in both dietary treatments, Control (CTRL) and Early (EARLY). The cod microdiet started to be introduced in the two treatments at 20 DPH and 12 DPH, respectively. Dry feed was delivered through the tanks via automatic feeders. Sampling points occurred at 12, 20, 43, 60, and 74 DPH, and zootechnical parameters were analyzed, including standard length (SL) and wet weight (WW).

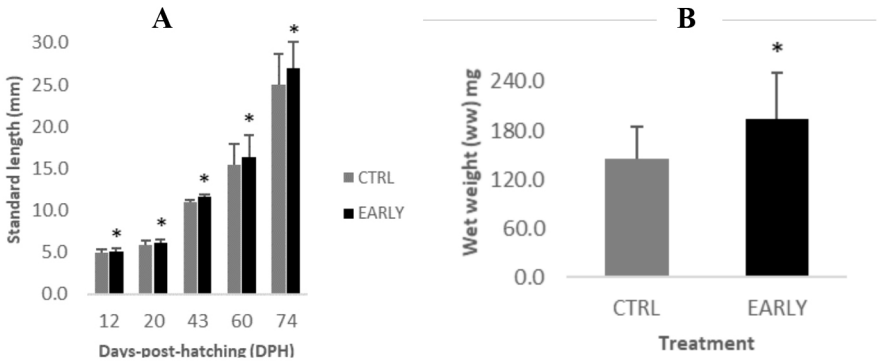


Figure 1. Standard length (A), at different sampling points; and wet weight (B), at 74 DPH, of cod larvae. Results are expressed as mean ± standard deviation. (*) represents significant differences between dietary groups ($p<0.05$).

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## Results

At every sampling point, the early introduction of a micro diet (EARLY) had a significant impact ($p<0.05$) on SL (mm) (Figure 1 A), and at 74 DPH on WW (mg) (Figure 1 B), compared to the control (CTRL) treatment. Relative growth rate around (3%/day) and survival rate from 20 to 74 DPH (13.4% CTRL; 17.4% EARLY) did not show any significant effect of the early introduction of dry feed.

## Discussion

Results suggest that early dry feed introduction in a co-feeding system, 12 DPH, can be an effective strategy to enhance growth performance on Atlantic cod larvae, both in standard length and wet weight, without compromising survival rates, comparing to CTRL group. This improvement in growth in the EARLY dietary treatment, is a promising result and can be helpful for the commercial aquaculture, since standard length can be a good indicator of future juvenile quality. While these results are encouraging, it is necessary to incorporate more detailed analyzes, including health markers and skeletal deformities, in order to have a more comprehensive view of larvae and juvenile quality.

## Acknowledgments

This work is part of the E!219 EarlyCOD project, supported by the EUROSTARS-3 program and co-financed by the European Union, Portugal and Norway, through grant 17205 (Algarve 2030: ALGARVE-FEDER-01006200; and COMPETE 2030: COMPETE2030-FEDER-01006200) in the framework of Portugal 2030, and through the Norwegian Research Council.

# IMMUNO-NUTRITION IN WEANING DIETS: THE USE OF PAECILOMYCES VARIOTII (PEKILO®) FOR GILTHEAD SEABREAM (*Sparus aurata*) LARVAE: EFFECTS ON GROWTH PERFORMANCE AND LARVAL HEALTH

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**Introduction**

Immunonutrition strategies are increasingly recognized as effective tools for enhancing disease resistance and post-stress resilience in farmed fish. One of those strategies involves the use of alternative ingredient, either protein- or lipid-based, that include bioactive compounds to improve fish health and robustness. Among the different alternative ingredients with bioactive compounds, single-cells proteins (SCPs) cover the different characteristic, as they combine readily digestible protein with bioactive compounds that support health, making it a nutrient-dense addition to aquafeeds. Among the different SCPs available, PEKILO® is a single-cell protein produced by cultivating the filamentous fungus *Paecilomyces variotii* on agricultural or forestry by-products. Animals fed PEKILO®-based diets exhibit better feed conversion efficiency, and they demonstrate higher survival rates under typical farming conditions. Moreover, the β-glucans and nucleotides in PEKILO® act as immunostimulants, enhancing the expression of pro-inflammatory cytokines and chemoattractant molecules and thereby strengthening the innate immune response of fish, including Atlantic salmon (*Salmo salar*) (Hooft et al., 2024; Mesah et al., 2025). However, the effect of this ingredient in marine warmwater fish, particularly during the early-development stages, when rapid growth coincides with immune system development—remain poorly understood. Therefore, the present study aimed to evaluate the inclusion of PEKILO® in weaning diets for gilthead sea bream larvae, with the goal of improving both growth performance and health status during this critical life stage.

**Material & Methods**

For that purpose, four different weaning diets were formulated diets with increasing levels of PEKILO® (0, 5, 10, and 15% inclusion) and the effect on larval growth performance, survival, stress resistance, and disease resistance against *Vibrio anguillarum* was studied. The experiment was conducted with 28 days post hatching (dph) larvae fed over 18 days in triplicates. Larvae were assigned to 12 fiberglass cylinder tanks with conical bottoms (170l), at 890 larvae/100L. Tanks were supplied with filtered seawater at an increasing debit from 1 L/min at the beginning of the experiment to 2 L/min at the end of the experiment and continuous aeration during the entire experiment. Water temperature was 22.2 ± 0.8 ºC and the dissolved oxygen was 6.57 ± 0.1 g/L. Larvae were hand-fed every 45 min from 8:00 am to 8:00 pm for 18 days until 47 dph. Photoperiod was 12:12 L:D and larvae mortality was daily registered by individually counting the dead larvae. At the end of the feeding period, two different tests were conducted with groups of thirty larvae from each tank (triplicates for each experimental diet): i) a stress test (2 min of air exposure) ii) a challenge test against infection with a sublethal dose of *Vibrio anguillarum* ($10^5$ CFU/ml). Mortality was recorded for 48 h, and the relative percentage of survival (RPS) was calculated at the end of the challenge.

At the beginning and at the end of the experiment, samples of whole larvae were taken to analyze proximal composition (crude protein, crude lipid, ash, moisture) and whole-body fatty acid and amino acid profiles. Whole larvae samples were also taken for histological studies. At the end of the feeding period and after challenge test, samples of whole larvae were also taken for analysis of relative expression of immune-related genes through real-time quantitative PCR (RT-qPCR) analysis. The analyzed genes included interleukin-1β (*il1b*), interleukin-10 (*il10*), tumor necrosis factor-α (*tnf-a*), nuclear factor kappa β subunit 2 (*nfk□2*) caspase 3 (*casp3*), cluster of differentiation 4 (*cd4*), cluster of differentiation 8 (*cd8*) and major histocompatibility complex II (*mhcII*) and using *b-actin* as housekeeping. Relative expression was calculated by the $2^{-\Delta\Delta Ct}$, where ΔCt was determined by subtracting the housekeeping from the Ct value of the target gene described by Livak and Schmittgen (2001).

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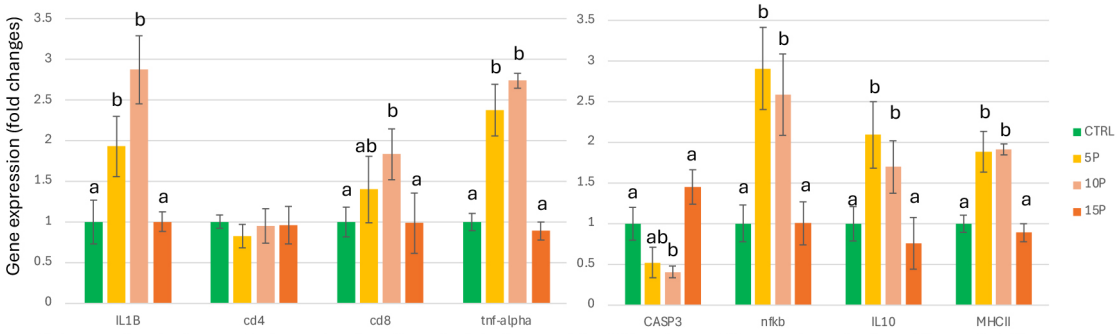
**Fig. 1**. Immune-related gene expression of whole larvae of Sparus aurata after 48 of challenge test against *V. anguillarum* and fed with different levels of *P. variooti* in weaning diets




Values are referred to the normalized expression of gene from animals feeding control diet. Different letters within a gene denote significant differences P<0.05

## Results

Results indicated a dose/dependent effect of *P. variotii* and could effectively use up to 10% of PEKILO® in diet without negatively affecting growth performance and survival, the inclusion of 15% of PEKILO® significantly ($p<0.05$) affecting larvae growth both in terms of weight and length. Larvae fed with higher levels of PEKILO® (15%) showed a significant ($p<0.05$) decrease resistance to pathogen in terms of survival, but had no effect on survival after stress test. Increasing levels of *P. variotii* did not affect whole-larvae proximate composition but did affect their fatty acid content, by increasing the n-6 fatty acids in whole larvae, and specifically the linoleic (18:2n-6) content in whole larvae. The inclusion of PEKILO® in seabream larval weaning diets improved the response of immune-related genes after 48h of infection with *V. anguillarum*, by increasing the relative expression of genes implied in the immune response against pathogens, such as *il1b*, *il10*, *tnf-a*, *nfk□2* or *mhcII*. However, the inclusion of 15% of *P. variotii* meal in larval diets failed to induce a proper response to the infection with *V. anguillarum* (Fig. 1).

This study demonstrates the potential of *Paecilomyces variotii* fungal meal as a sustainable and functional ingredient in weaning diets for gilthead sea bream. Levels of inclusion up to 10%. improve larval growth and the response of larvae to infection with *Vibrio anguillarum*, but higher level of inclusion (15%) induced negative effects on larval survival after challenge test against pathogen, denoting a dose-dependent effect.

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## Acknowledgments

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performed at ULPGC-FITU (Feed Ingredients and Additives Testing Unit) research infrastructure, under grant agreement PID

# INTEGRATED CHEMICAL AND ECOTOXICOLOGICAL ASSESSMENT OF THE IMPACT OF MILITARY ACTIVITIES ON AQUATIC ECOSYSTEMS IN UKRAINE

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The investigation is focused on evaluating the ecotoxicological status of aquatic ecosystems within conflict-impacted regions of Ukraine. This assessment is based on physicochemical and biological analyses of water and bottom sediment samples from 12 selected water bodies. Preliminary chemical analyses indicated that concentrations of heavy metals (Ni, Pb, Cr, Cu, Hg, Cd, Zn) and 15 polycyclic aromatic hydrocarbons (PAHs) in surface waters were below the detection limits of the analytical methods employed. Sediment analyses revealed low concentrations of heavy metals within regulatory thresholds and slightly elevated PAH concentrations in certain locations, suggestive of legacy contamination associated with military activity. Nutrient and organic matter content in sediment eluates exhibited substantial spatial variability, with Lake Sasyk (Odessa region) demonstrating the highest levels of total carbon (11.47 mg/L), total organic carbon (4.91 mg/L), and total phosphorus (0.055 mgP/L). These findings suggest significant organic and nutrient loading, likely attributable to anthropogenic sources. Radiological analyses identified only natural radionuclides at background levels; artificial radionuclides (e.g., $^{137}$Cs) were below the detection limits of the analysis. Ecotoxicological assessments employed a battery of bioassays involving multiple trophic levels, including green alga *Desmodesmus communis*, the higher plant *Lepidium sativum*, crustaceans (*Daphnia magna, Thamnocephalus platyurus*), ostracods (*Heterocypris incongruens*), and 8zebrafish (*Danio rerio*). The analysis revealed that several samples elicited biologically significant effects, including increased mortality, sublethal growth inhibition, oxidative stress, and behavioral alterations, despite the absence of detectable concentrations of the tested contaminants. It is noteworthy that *D. rerio* exhibited developmental impairments, and crustaceans demonstrated acute and chronic toxicological responses. These outcomes underscore the potential presence of bioavailable, mixture-based, or unidentified toxicants that elude standard chemical analyses. The findings underscore the limitations of relying exclusively on chemical profiling in environmental risk assessment, particularly in post-conflict contexts. The observed species-specific and endpoint-specific responses emphasize the necessity of incorporating integrative ecotoxicological approaches that account for physiological and ecological traits to accurately characterize environmental health and inform remediation strategies.

Acknowledgement
This work was funded by NATO Science for Peace and Security Programme “Creating a Strategy for Assessing and Restoring War-affected Aquatic Ecosystem” (project No G6085).

# PROTEIN HYDROLYSATES FROM PORCINE BLOOD FOR AQUACULTURE FEED: INFLUENCE OF HYDROLYSIS TIME ON THEIR BIOACTIVE PROPERTIES

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**Introduction**

The valorisation of meat industry by-products, such as pig blood, represents an efficient strategy to generate functional ingredients for animal nutrition that would contribute to the sustainability of the meat and aquaculture sectors simultaneously. Blood is a rich source of high biological value proteins, whose enzymatic hydrolysis allows the production of peptides with bioactive properties (Bah et al., 2013). These compounds can contribute to the health and behaviour of aquatic organisms when incorporated into functional feeds, while promoting the circular economy of the agri-food sector (Salin et al., 2018).

In this context, the aim of this study was to optimise the sequential hydrolysis time of porcine blood with commercial proteases (Alcalase 4. 0 L and Protana™ Prime), evaluating parameters such as the degree of hydrolysis and peptide profile, as well as the effects on the inhibition of enzymes of metabolic and physiological interest (DPP-IV, NEP, TACE, MGL), completed with *in silico* peptidomic studies to predict the potential functionality and characteristics of the identified peptides.

**Materials and Methods**

Porcine blood hydrolysis was performed according to Moreno-Mariscal et al. (2025). Briefly, after an ultrasound pretreatment (1 hour, 35 kHz) and diluted 1:1 with bidistilled water, 2% Alcalase 4.0 L (endoprotease) was added to all hydrolysates at 65ºC at different times: 2h (APP2), 6h (APP6) and 24h (APP24). After that, 5% Protana™ Prime (exoprotease) (overnight, 55ºC) was added to each hydrolysate and then finally inactivated by heating. The degree of hydrolysis was determined using OPA reagent and the peptide profile of hydrolysates was studied through membrane ultrafiltration with different cut-offs (30, 10 and 3 kDa). The inhibition of dipeptidyl peptidase IV (DPP-IV) and neprilysin (NEP) enzyme activities were assessed to evaluate the hypoglycemic potential, while the inhibition of tumor necrosis α-converting enzyme (TACE) and monoacylglycerol lipase (MGL) enzymes was measured to investigate the anti-inflammatory potential of the peptides present in the different hydrolysates. Finally, peptides were identified by tandem mass spectrometry (LC-MS/MS) and subjected to an *in silico* analysis to predict its potential to be bioactive (Peptide Ranker tool), allergenicity (AllerTOOP v.2.0 tool), toxicity (ToxinPred tool) and cell penetrating probability (CPPpred tool).

**Results and Discussion**

Enzymatic hydrolysis with Alcalase 4.0L does not necessarily imply a higher degree of hydrolysis (DH). Statistical analysis showed that the APP6 (23.43±0.95%) hydrolysate had the lowest DH value, while APP2 (25.50±0.46%) and APP24 (24.23±1.48%) showed no significant differences between them. However, peptide profile analysis revealed that longer Alcalase 4.0L treatment time was associated with a higher proportion of low molecular weight peptides. The high proportion of peptides smaller than 3 kDa (~80%) can be attributed to the high exoprotease activity of the enzyme Protana™ Prime. In vitro enzyme inhibition assays indicated differences in peptide profiles between the different hydrolysates. While no significant differences were observed in DPP-IV enzyme inhibition, variations were identified in NEP inhibition, where inhibitory activity appeared to be correlated with larger peptide size. In the case of TACE inhibition, APP2 (80.68±4.43%) showed the highest percentage of inhibition, followed by APP24 (66.18±7.29%) and APP6 (57.49±6.03%). In contrast, MGL inhibition was most pronounced in APP24 (64.76±7.54%), followed by APP6 (48.87±2.33%) and finally APP2 (33.13±0.11%), suggesting that the responsible peptides might be of smaller molecular size. Mass spectrometry analysis (LC-MS/MS) identified 16 peptide sequences in APP2, 3 in APP6 and 7 in APP24, all with a PRR value > 0.75, indicating a high bioactive potential. Of these sequences, 12 had no allergenic potential and only one showed signs of toxicity.

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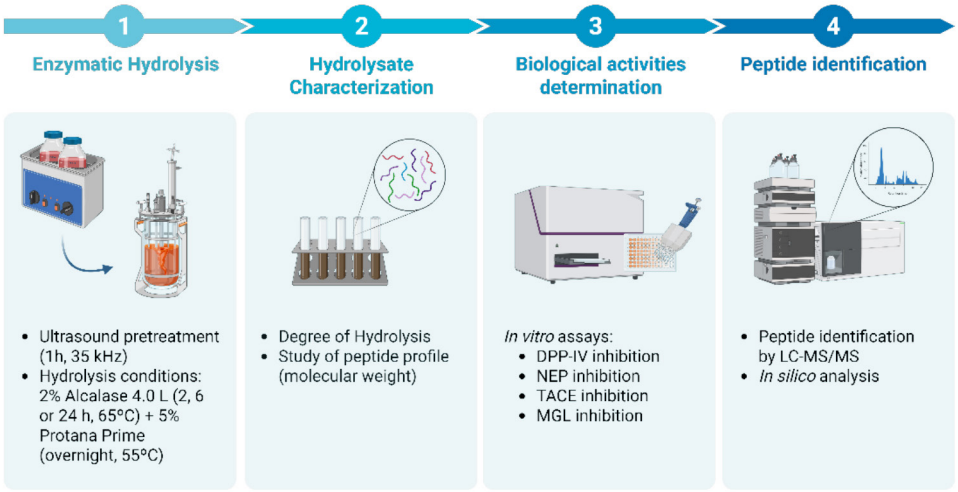


**Figure 1.** Graphical summary of the production and characterization of bioactive peptide-rich hydrolysates from porcine blood (created in https://BioRender.com).

## Conclusions

In conclusion, the hydrolysis of porcine blood using Alcalase 4.0L and Protana™ Prime is an effective strategy for the generation of small molecular size bioactive peptides with low risk of toxic or allergenic effects, constituting an important source of new ingredients for the formulation of aquaculture functional feeds.

## Funding

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# OLIVE BIOACTIVES AS A CIRCULAR BIOECONOMY SOLUTION TO INTESTINAL INFLAMMATION IN EUROPEAN SEABASS *Dicentrarchus labrax*

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## Introduction

Inflammation in aquaculture species can arise from various stressors such as suboptimal diets, environmental fluctuations, and pathogen exposure. Chronic or unresolved intestinal inflammation impairs nutrient absorption, compromises immune function, and ultimately hinders growth performance and overall fish welfare. Exploring functional ingredients with anti-inflammatory properties offers a promising strategy to support intestinal integrity and enhance resilience in farmed fish. Spain, by far the world's largest olive oil producer, generates large volumes of by-products, including olive pomace containing valuable bioactives like triterpenoids. Therefore, olive-derived by-products have great potential to offer a circular, eco-friendly approach to mitigate inflammation, and enhance productivity and animal well-being, improving overall aquaculture sustainability. To test this hypothesis, we used a protocol developed by Sparos Lta. to chemically induce intestinal inflammation in European seabass using dextran sodium sulphate (DSS).

## Materials and methods

The trial comprised 2 phases. In phase I, the whole fish population was fed a diet containing DSS for 15 days to induce a leaky gut and inflammatory response. Then, six fish were sampled to assess the intestinal inflammatory status, and the remaining were sorted and homogeneously distributed into quadruplicate 500L tanks, with 45 fish/tank, supplied with recirculated and aerated water. Phase II consisted in hand feeding the fish (start weight: 35.0±2.1 g) the experimental treatments to visual satiety, 2-3 times per day, for 61 days. A control diet (CTRL) was formulated mimicking a practical low fishmeal diet, with 44% crude protein, 17% crude fat level and 21.3 MJ/kg gross energy. Two additional treatments were prepared by supplementing the same formulation with 0.2% or 0.4% of an additive containing olive pomace extract (OE). Water quality was monitored throughout the trial and kept at good levels; average temperature was 19.5±0.3ºC. At the end of phase II (day 77), all fish were weighed, 6 fish/tank were pooled for whole-body composition analysis, and 3 individual fish/tank were sampled for subsequent analysis, to assess growth, feed utilization and intestinal health status (recovery from inflammation).

## Results and discussion

At the end of phase II, fish reached, on average, 87.6g, 88.2g or 102.5g in the CTR, OE 0.2% or OE 0.4% treatments, respectively. This represented a significant 17% higher growth in fish fed OE 0.4% during the recovery period. Feed intake was not affected by OE dietary inclusion. As a result, FCR was also significantly improved (by 11.7%) in fish fed the OE 0.4% diet. Moreover, this treatment had a higher whole-body retention (% of intake) of protein, fat and energy (Fig. 1).

Intestinal gene expression was analyzed by RT-qPCR in the general fish population on day 15 (end of the DSS administration period), and on day 77 in each treatment to verify the degree of recovery from intestinal inflammation. A panel of genes was selected to evaluate the inflammatory response (Fig. 2) and integrity (Fig. 3) of the intestinal epithelium. Results were normalized by the average expression of *ef1a*, *bact* and *gapdh*.

All results shown below correspond to the mean ± SEM. Bars with different letters denote significant differences, assessed by one-way ANOVA and Fisher's LSD ($P<0.05$).

Intestinal expression of inflammatory mediators (*il6, il8, il10, il1β, tnfa* and *nfkb2*) was significantly reduced between day 15 and day 77, indicating that fish were able to at least partly recover from inflammation by the end of phase II (Fig. 2). Additionally, the expression of *il6, il10* and *tnfa* was significantly lower in OE 0.4% than in the CTRL on day 77, suggesting that the lowest inflammatory status was achieved in this treatment.

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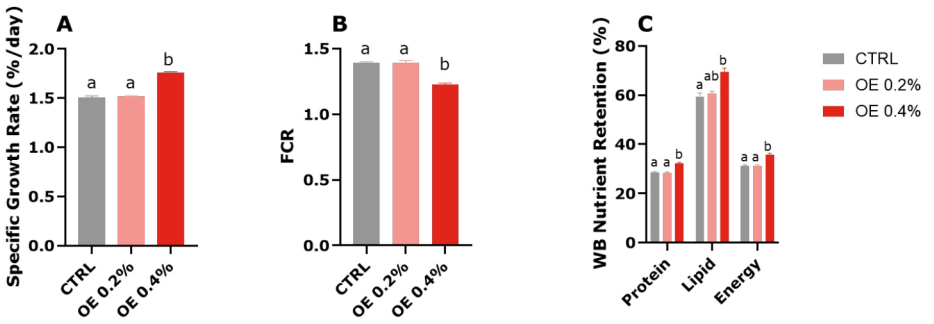


Fig. 1. Zootechnical performance at the end of phase II. A- Specific growth rate (%/day). B- Feed conversion ratio (FCR). C- Whole-body nutrient retention (% of intake).

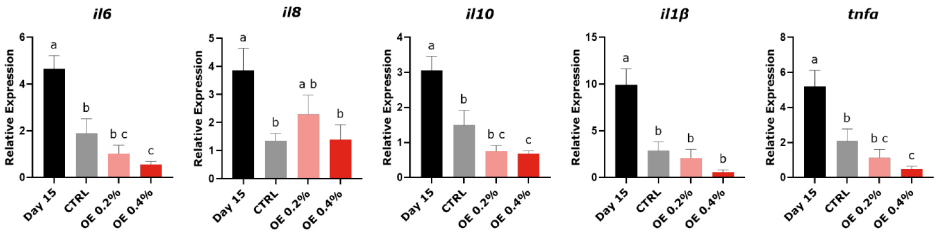


Fig. 2. Relative expression of selected inflammatory mediators at the end of phase I (day 15) and on day 77 in the three experimental treatments fed during phase II.

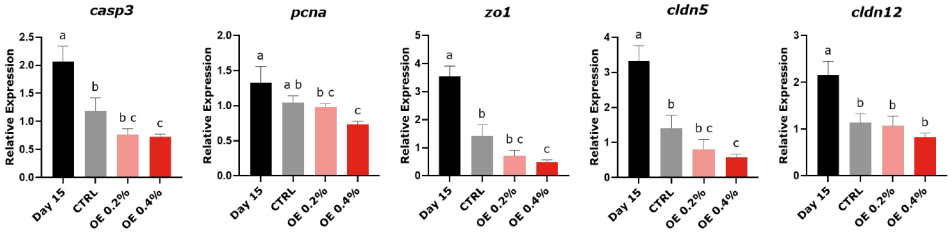


Fig. 3. Relative expression of selected intestinal integrity markers at the end of phase I (day 15) and on day 77 in the three experimental treatments fed during phase II.

Markers of apoptosis (*casp3*) and cellular proliferation (*pcna*), and genes coding for tight junction proteins (*zo1*, *cldn5* and *cldn12*) were significantly elevated on day 15 (Fig. 3). This suggests that a compensatory mechanism was activated to counteract the loss of intestinal barrier integrity. Conversely, the significantly lower expression of these genes (except *cldn12*) in the OE 0.4% treatment, relative to the CTRL, could be a sign of improved membrane integrity and higher recovery from DSS-induced damage.

These results clearly indicate a positive effect of the OE at 0.4% dietary inclusion level in alleviating intestinal inflammation in European seabass. Moreover, the faster and/or higher recovery from the DSS-induced disruption of intestinal integrity and inflammation was associated with significant improvements in fish growth, feeding efficiency and whole-body nutrient retention. Results are still pending on histological examination of the intestine, and plasma and feces inflammation markers, which are expected to further support the potential of triterpene-rich OE as a practical solution for mitigating intestinal inflammation challenges in fish farming.

# SUPEROXIDE DISMUTASE ACTIVITY AND ISOENZYME PATTERN IN THE DIGESTIVE GLAND OF SUBADULT AND JUVENILE WILD *Octopus vulgaris* OVER A 24-HOUR PERIOD

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## Introduction

Behavior and physiology in living organisms are regulated by endogenous rhythms that allow them to anticipate to predictable changes in their environment, which is essential for survival (Häfker et al., 2023). These oscillations are directly related to the daily rhythm of activity of antioxidant enzymes (Wilking et al., 2013), making them excellent bioindicators of animal lifestyle and welfare. Among antioxidant enzymes, the superoxide dismutase (SOD) family, which protects against oxidative damage by catalyzing the dismutation of superoxide anion radicals into oxygen and hydrogen peroxide, has been reported to play a key role in physiology and disease. The condition of the digestive gland in *Octopus vulgaris* is considered a good indicator of feeding habits and welfare since it is responsible for digestion, absorption and storage of nutrients. In this study, we investigate both the specific SOD activity and the SOD isoenzymes present in the digestive gland (DG) of wild subadult and recently settled juvenile *O. vulgaris* over a 24-hour cycle using espectrophotometric and electrophoretic analysis.

## Materials and Methods

*Collection of material*: *O. vulgaris* individuals were collected using an artisanal fishing boat from the Vilanova i la Geltrú fishing fleet (NW Mediterranean). A total of 35 subadults were collected between 5:30 and 10:30 hours of October 9th, 2023, using octopus pots (Sauer et al., 2021) placed at 5–27 m depth in the sampling area. Size of subadults was selected aiming to sample non-mature individuals to avoid adding unknown sexual maturity factors that can potentially influence internal rhythms. A total of 35 juveniles were collected between 06:30 and 11:00 h of November 11th, 2023, using experimental juvenile octopus' collectors (Escolar et al. 2021) placed at 5–15 m depth on sandy bottoms of the same area. Each collecting day, both subadults (mean weight: 587 ± 111 g; range: 408–850 g) and juvenile (mean weight: 0.427 ± 0.622 g; range: 0.183-3,428 g) were kept in containers onboard, transported to aquarium facilities of the ICM-CSIC, located at 50 km, and placed in rearing tanks by 13:00 h. All individuals were maintained in the aquarium facilities of the ICM provided by an open seawater system under natural photoperiod and water temperature (23.9 °C for subadults, and 17.2 °C for juveniles). To avoid agonistic interactions, individuals were distributed in the tanks based on their size, and for subadults the sex was also considered. Light intensity at tank bottom was 80-90 lux for subadults and 15 lux for juveniles. Natural sunrise and sunset during the experiment with subadults were 7:57 and 19:19 local time, and 07:45 and 17:45 local time for juveniles, respectively. No food was provided.

*Sampling for endogenous rhythms analysis*: The day after they were captured, five individuals were sampled every 4 hours across seven time points starting at 12:00 h (t0 (midday)), 16:00 h, 20:00 h (t4, t8 (afternoon, evening)), 24:00 h (t12, (midnight)), 04:00 h, 08:00 h (t16, t20, (early morning, sunrise)) and 12:00 h (t24 (midday, after 24h)). Special care was put into avoiding any light source during night sampling hours in the aquaria facilities, and operations inside the aquaria facilities were performed using infrared lights. Digestive glands (DG) were dissected after the death of the individuals and stored at -80 °C until analysis. Animal welfare procedures followed EU guidelines (Directive 2010/63/EU) and cephalopod-specific recommendations (Fiorito et al., 2015).

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*Superoxide dismutase enzyme assay:* SOD specific activity was measured by the ferricytochrome c method using xanthine/xanthine oxidase as the source of superoxide radicals (McCord and Fridovich, 1969). Enzyme assay were carried out at 25 °C using a PowerWaveX microplate scanning spectrophotometer (Bio-Tek Instruments, USA) and run in duplicate in 96-well microplates (UVStarR, Greiner Bio-One, Germany).

*Analysis of SOD isoenzymes*: For the separation of SOD isoenzymes, nondenaturing PAGE was performed on 10% acrylamide slab minigels (MiniProtean II, Bio-Rad). SOD isoenzymes were detected in gels by the photochemical NBT (nitroblue tetrazolium) staining method (Beauchamp and Fridovich, 1971).

**Discusion**

The daily dynamics of the specific activity of SOD in the digestive gland of *Octopus vulgaris* shows a maximum in the early morning (4:00 h) in subadults and juveniles, and only in juveniles an increased activity was also found near the midday. Regarding SOD isoenzymes, the daily dynamic is similar than that reported for specific activity, but whereas in subadults only the Cu/Zn-SOD was present in juvenile individuals both Cu/Zn-SOD and Mn-SOD were identified. Cu/Zn-SOD and Mn-SOD are the two main SOD isoenzymes present in eukaryotes, the first found in cytoplasm and the second in mitochondria (Perry et al., 2010). *Octopus vulgaris* is a meropelagic cephalopod species, with juveniles suffering a drastic change from an active planktonic life to a more sedentary benthic stage during the settlement period, which involves important physiological and morphological changes that might be responsible for these changes in the SOD activity and isoenzyme pattern. Although subadult and adult *Octopus vulgaris* are known to be mostly nocturnal predators, the predatory habits of juveniles in the wild are unknown (Escolar et al., 2024). Whether or not the present results reflect different predatory habits in juveniles requires further ecological and physiological studies.

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**Acknowledgements**

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# PROTEOMIC ANALYSIS REVEALED POTENTIAL HEALTH EFFECTS OF FUCOIDAN FROM SUGAR KELP (*Saccharina latissima*) IN VACCINATED ATLANTIC SALMON (*Salmo salar*)

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## Introduction

Salmonid products represent ~20% of global seafood exports by value. Projections from the Food and Agriculture Organization indicate that fish farming will continue to expand in the coming decades (FAO, 2024). However, high mortality rates – driven by multi-stressor conditions (e.g., infectious diseases and environmental problems), remain a significant challenge in major salmonids producing countries such as Norway and Chile. To address this problem, the combination of vaccines and functional feeds with bioactive additives is a strategy that has gained interest in recent years. In the Resilient salmon project (RCN 294821) we aim to improve the immune response of Atlantic salmon (*Salmo salar*) against *Tenacibaculum maritimum*, a bacterial skin pathogen that threatens Norwegian aquaculture, and for which no commercial vaccines currently exist.

To achieve our goal, we combined a specific nutritional program with novel feeds containing fucoidan from sugar kelp (*Saccharina latissima*) during freshwater (FW) stage, along with a commercial pentavalent vaccine that do not contain *T. maritimum* antigens. Previously, we have described how fucoidan fractions are able to modulate the expression of immune-related genes in salmon head kidney cells (Michalak et al, 2023). We also reported that vaccinated Atlantic salmon fed fucoidan up-regulated and increased the protein level of antimicrobial peptides (e.g., *hepcidin* and *cathelicidin2*) in head kidney after stimulation with inactivated *T. maritimum* (by intraperitoneal injection) in seawater (SW) stage. However, further characterization is needed to understand the bioactive properties of fucoidan in fish. Therefore, in the current study, a proteomic approach was used to analyze the potential health effects of this compound in the distal intestine and head kidney of Atlantic salmon.

## Materials and Methods

At the NMBU's Center for Fish trials, pre-smolt Atlantic salmon were vaccinated with ALPHA JECT micro 6 vaccine (PHARMAQ). Then, fish were distributed into 300 L tanks (45 fish per tank) supplied with recirculated FW. Each tank (in duplicate) was assigned to a commercial-like diet (Ctr) or an experimental diet containing 0.2% fucoidan extract from sugar kelp (FD) for four weeks. After this period, 35 fish per tank were transferred to the SW facilities at the Norwegian Institute for Water Research (Solbergstrand, Norway) and fed the Ctr for the reminding experiment. After four weeks in SW, fish were intraperitoneally (IP) injected with inactivated *T. maritimum*. Samples from distal intestine (DI) and head kidney (HK) were collected from six fish per tank at different time points: end of FW stage (S2), after four weeks in SW (S3), one day post IP injection (S4) and seven days post IP injection (S5). Total proteins were obtained from each sample and then quantified with the BCA method. To perform a proteomic analysis, six protein samples from each organ, dietary group and sampling were digested and analyzed through mass spectrometry by the Proteomics Unit at University of Bergen (PROBE) according to Mensah et al (2025). Raw data files were processed by Perseus v.1.5.6.0 (MaxQuant). Thereafter, Limma v.3.61.9 was used in RStudio to determine the differentially modulated proteins between dietary groups. A contrast matrix between the FD and Ctr was created, and the significantly modulated proteins (p-value < 0.05) were determined. Modulated proteins (LAP: low abundance proteins; HAP: high abundance proteins) were used for a functional analysis by KEGG mapper (Pathways and Brite databases) according to Purushothaman et al (2024). Our study presents the results in which four or more hits were detected per term.

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**Table 1**. Modulated proteins in FD group compared to fish fed Ctr.

| Organ | Sampling | LAPs | HAPs | Total |
|---|---|---|---|---|
| DI | End of FW stage (S2) | 113 | 28 | 141 |
| | 4 weeks in SW (S3) | 61 | 168 | 129 |
| | 1d post IP injection (S4) | 250 | 271 | 521 |
| | 7d post IP injection (S5) | 181 | 361 | 542 |
| HK | End of FW stage (S2) | 223 | 383 | 606 |
| | 4 weeks in SW (S3) | 386 | 241 | 627 |
| | 1d post IP injection (S4) | 217 | 184 | 401 |
| | 7d post IP injection (S5) | 347 | 318 | 665 |

**Results and Discussion**

Overall, the comparison of proteomic data from fish fed FD and Ctr revealed that HK had a higher number of modulated proteins (LAPs and HAPs) than DI (Table 1). In addition, after the functional analysis of these proteins, DI from S2 had LAPs related to glycosaminoglycan binding proteins, whereas HK at the same sampling point showed HAPs linked to transcription machinery, cell adhesion molecules, cytoskeleton proteins, cytokine receptors, chaperones and folding catalysts (among others). This suggests that the dietary inclusion of fucoidan can modulate a primary lymphoid organ such as HK, which is involved in the coordination of local and systemic immune responses.

After the stimulation with inactivated *T. maritimum*, both DI and HK had HAPs associated with CD molecules and transporters at 1d post IP injection (S4). Also, HAP-related pathways such as endocytosis and antigen processing and presentation were detected in HK, while neutrophil extracellular trap formation, NOD-like receptor signaling, as well as complement and coagulation cascades were detected in DI. By contrast, S5 showed LAPs in DI linked to transcription factors and cytokine receptors, and LAPs in HK such as chaperones and folding catalysts, proposing a regulation of the response seven days post IP injection.

**Conclusions**

The inclusion of fucoidan in functional feeds for vaccinated Atlantic salmon pre-smolts showed the modulatory capacity (at the phenotypic level) of this compound in DI and HK. Interestingly, after seawater transfer and 1d post-IP stimulation with inactivated *T. maritimum*, fish previously fed fucoidan in FW exhibited enhanced health-related responses in SW, which declined 7 days post-IP stimulation.

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# EFFECT OF THE DIETARY INCLUSION OF A HYDROLYSED AND FERMENTED ALGAL BLEND ON GROWTH AND INTESTINE FUNCTIONALITY IN JUVENILE *Chelon auratus*

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## Introduction

The rising costs of fishmeal and fish oil have created the need to search for new feed ingredients, allowing these feedstuffs to be replaced by more sustainable and costeffective alternatives while maintaining the nutritional value of aquafeeds. In this regard, algae appear as a promising option for inclusion in fish feed, due to their valuable nutritional value, making them a great source of protein, omega-3 polyunsaturated fatty acids, antioxidants, pigments, vitamins, and minerals, which can improve the health and development of animals (Galafat *et al*., 2024). In addition, species diversification can increase the competitiveness of the aquaculture sector. In this way, the golden grey mullet (*Chelon auratus*) emerges as a promising species due to its low dietary protein requirements and its high fillet quality (García-Márquez *et al*., 2024). However, the nutritional value of algae may be limited by the presence of a thick and complex cell wall, which hinders the bioavailability and accessibility of nutrients and intracellular compounds. Therefore, biotechnological pretreatments, such as enzymatic hydrolysis or microbial fermentation, become essential, as they have been shown to improve nutrient bioavailability (Vizcaíno *et al*., 2024). For this reason, the aim of this study was to evaluate the dietary inclusion of a hydrolysed and fermented algal blend on the growth and digestive functionality in *Chelon auratus* juveniles.

## Material and Methods

The algal blend used contained 30% *Arthrospira* sp., 20% *Chlorella* sp., 10% *Microchloropsis* sp., 10% *Tisochrysis* sp., and 30% *Ulva* sp. It was supplied by the company LifeBioencapsulation S.L. (Almería, Spain) and stored at -20 °C until use. A sequential biotechnological process was carried out, first subjecting the algal biomass to enzymatic hydrolysis with a mixture of hydrolytic enzymes (carbohydrases, cellulase, and proteases) during 5 h, and then followed by microbial fermentation using $10^9$ CFU/mL of *S. cerevisiae* and $10^9$ CFU/mL of *B. subtilis* for 36 h.

A 62-day feeding trial was conducted at the Marine Culture Facility of the University of Cádiz. For this purpose, two experimental diets, L50 and L100, were formulated to replace 50% and 100% of the fishmeal and fish oil, along with a control diet (CT) including 15% fishmeal and 5% fish oil without algal biomass. At the end of the trial, fish growth was analyzed, and biological samples were collected to evaluate the proximal composition of the muscle, digestive enzyme activities, and the ultrastructure of the intestinal mucosa.

## Results and Disc[illegible]

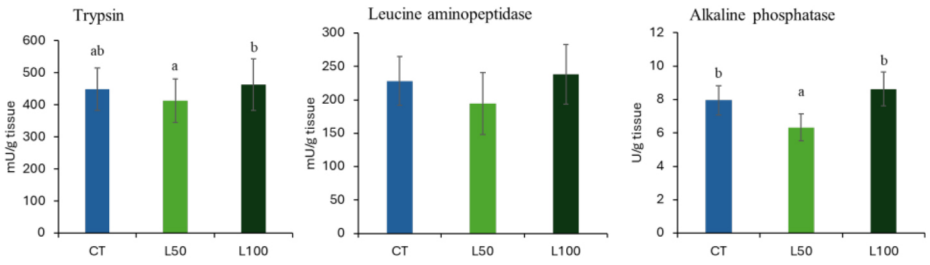


Figure 1. Digestive enzyme activities of *C. auratus* juveniles fed the different experimental diets.

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On the one hand, no significant differences were observed in growth performance among treatments. Similarly, the proximal composition of the muscle showed no differences, except for the total lipid content, which was higher in the L100 group. On the other hand, enzymatic activity assays showed that trypsin and alkaline phosphatase activities were significantly higher in the fish fed the L100 diet compared to the CT group (Figure 1), which could indicate an improvement in the digestive capacity of these fish. In addition, scanning electron microscopy (SEM) images showed a healthy intestinal mucosa, with no signs of damage in any of the treatments evaluated. Results obtained confirmed the suitability of total replacement of fishmeal and fish oil in practical diets for this fish species.

Acknowledgments

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# MONITORING THE GROWTH OF NILE TILAPIA (*Oreochromis niloticus*) USING ACOUSTIC TECHNIQUES

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## Introduction

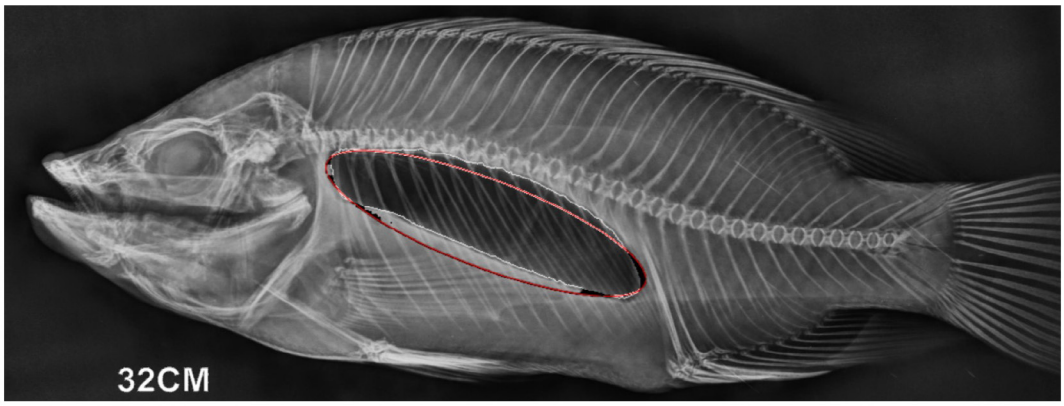
The Nile tilapia (*Oreochromis niloticus*) is the second most farmed fish species globally, typically reared under high-turbidity conditions that hinder direct visual monitoring. This study aimed to develop methods for non-invasive growth assessment using lateral target strength (TS) as a proxy for fish length in free-swimming individuals under production-like conditions.

## Data and Methods

Eighty-seven specimens were reared in controlled environments, and biometric measurements (length, weight, swim bladder dimensions) were obtained to define size groups. Lateral TS measurements were collected using a BioSonic echosounder (~200 kHz), yielding 30,477 individual traces. Two regression models relating mean group length to both mean and maximum TS values were fitted. Numerical simulations using the Method of Fundamental Solutions, based on swim bladder geometry derived from X-ray imaging, were performed to produce TS estimates comparable to experimental values.

## Results

Regression models achieved high correlations ($R^2 > 0.9$) between fish length and TS parameters. Simulated TS values showed consistency with measured data. An empirical equation was proposed to link TS and mean length, enabling average population size estimation from acoustic data. Findings demonstrate that lateral TS is a robust indicator for growth monitoring in *O. niloticus*, supporting its potential application for biomass estimation in aquaculture systems. Future research will address the application of this methodology across complete production cycles.

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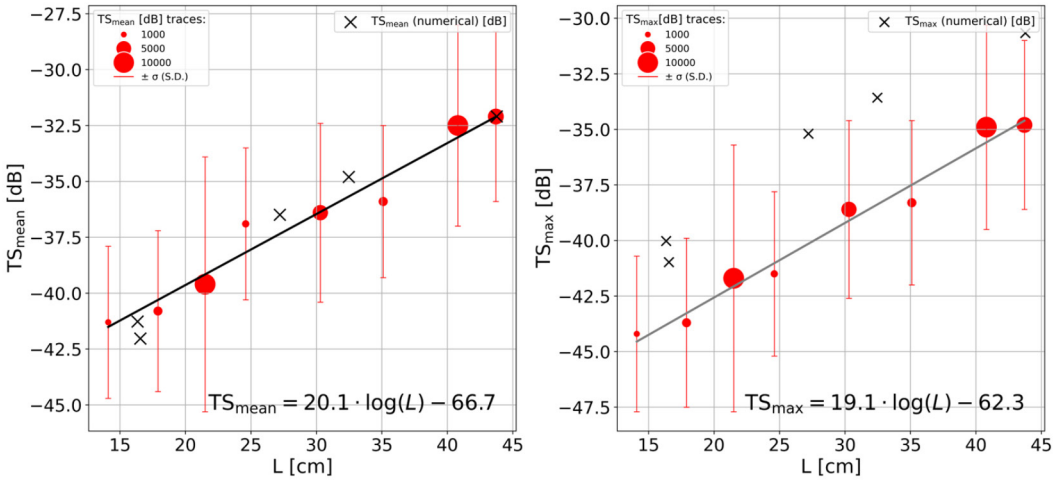
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# FIRST APPROACH FOR THE ACOUSTIC FEEDING MONITORING OF SHRIMP (*Penaeus vannamei*)

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## Introduction

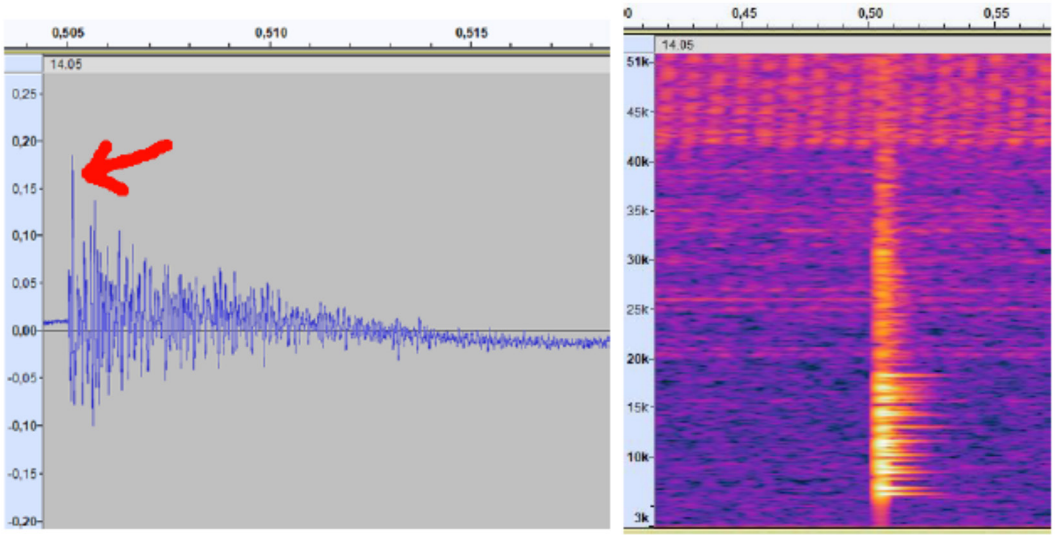
Feed management in *Penaeus vannamei* culture represents the main operational cost and a key sustainability challenge. This species produces characteristic short-duration "click" sounds while feeding on pellets, generated by the collision of its calcareous maxillae. Passive acoustic monitoring (PAM) offers a non-intrusive method to detect and characterize such feeding activity. This study aimed to record and analyze these clicks as a first step toward developing acoustic-based feeding control systems, and to explore the relationship between shrimp size and click sound pressure levels (SPL).

## Material and Methods

Forty-eight specimens were measured, weighed, and fed pellets of uniform size in a controlled laboratory tank. Feeding activity was recorded with a calibrated omnidirectional hydrophone system. SPL values were calculated from the recorded data, and spectral analysis was performed using Fast Fourier Transform of the signal.

## Results and Discussion

A total of 413 clicks were identified, with durations averaging 0.01 ± 0.003 s, SPLs typically between 140–144 dB re 1 $\mu$Pa, and maximum values around 168 dB. The clicks' frequency content ranged from 3 to 38 kHz. Direct oscilloscope measurements confirmed SPL estimates. The recorded sound levels were higher than those reported in previous studies, possibly due to methodological or environmental factors. This acoustic characterization establishes a foundation for future work aimed at optimizing feeding strategies by integrating PAM systems in shrimp aquaculture.

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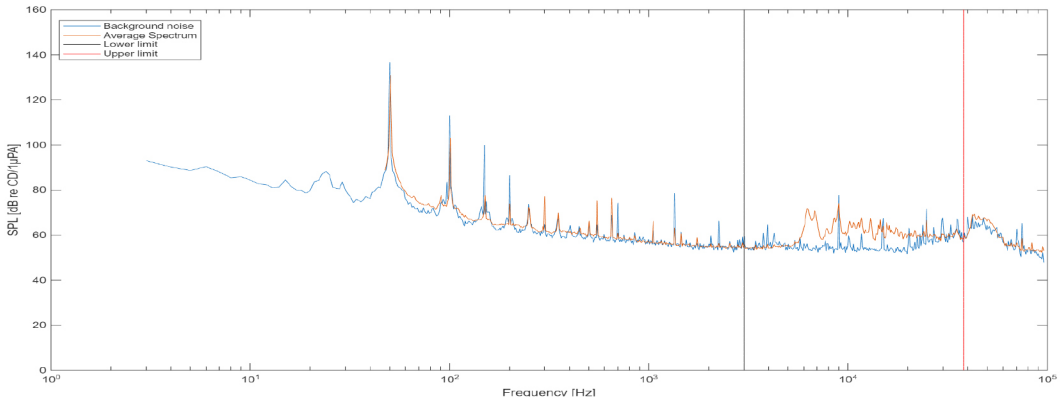
Background noise
Average Spectrum
Lower limit
Upper limit
SPL [dB re 0D/µPA]
Frequency [Hz]
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10^3
10^4
10^5

# LEGAL RESPONSES TO MICROPLASTICS IN EUROPE: AN INTERDISCIPLINARY REVIEW FROM A MARINE ECOLOGY PERSPECTIVE

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Microplastics (MPs) are increasingly recognized as a planetary boundary threat (Persson et al., 2022, p. 1513), severely impacting marine ecosystems, food chains, and human health. Despite extensive evidence on their ecological harm—from cellular damage in plankton to bioaccumulation in top predators (Bucci et al., 2020, p. 5)—Europe's regulatory framework remains fragmented and largely inadequate. This interdisciplinary review critically analyses the state of the art on marine microplastic pollution from a legal-ecological perspective, with a focus on European Union regulations and their implications for marine biodiversity protection and enforcement mechanisms.

We review scientific findings on microplastic dispersion, degradation, and toxicity in coastal and marine habitats (Kurniawan et al., 2024, p. 3; Bamigboye et al., 2024, p. 261), alongside legal instruments such as the Marine Strategy Framework Directive, REACH regulation, and the recent EU ban on intentionally added microplastics (Zuccaro et al., 2024, p. 2592). Drawing from jurisprudence across more than 30 jurisdictions (Varvastian, 2023, p. 636), we evaluate the emerging role of courts in filling governance gaps and driving state accountability.

Our findings highlight a mismatch between the complexity of microplastic pollution—particularly its invisible, diffuse, and transboundary nature—and the enforcement capacities of administrative and criminal law. We argue that while legal regimes recognize the environmental harm of plastics, current implementation lacks the ecological sensitivity and interdisciplinary coordination required for effective mitigation. Notably, cruise ship-related pollution and antifouling paint degradation are emerging as critical, yet understudied, legal blind spots (Compa et al., 2023, p. 12; Morelle-Hungría, 2022, p. 5).

We propose a multi-layered governance approach integrating marine ecological evidence with enforceable legal standards. Specific attention is given to the potential role of environmental criminal law and citizen science mechanisms to enhance compliance and monitoring efforts. This work contributes to the dialogue between marine science and environmental law, advocating for stronger regulatory instruments aligned with planetary boundaries and the precautionary principle.

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# BOOSTING SEA BREAM WELFARE AND WELLBEING USING PORCINE BLOOD HYDROLYSATE AS A FUNCTIONAL FEED INGREDIENT

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## Introduction

The escalating global demand for animal proteins has led to a substantial increase in the generation of animal by-products (ABPs) by the meat processing industry. Among these, porcine blood -constituting approximately 3% of the live weight of pigs- emerges as a promising substrate for its valorisation into high-value bioproducts through environmentally sustainable methodologies, thereby contributing to the advancement of circular economy practices (Toldrá et al., 2021). In this regard, sequential enzymatic hydrolysis has been identified as a key methodological procedure, as it not only enhances the digestibility of protein substrates but also facilitates the release of bioactive peptides and free amino acids. Accordingly, this approach holds significant potential for the development of functional feed ingredients tailored for aquaculture applications, with the dual aim of promoting aquatic animal health and welfare and reinforcing the environmental sustainability of aquaculture systems (Boyd et al., 2020). To achieve this goal, we evaluated *in vitro* the properties of a novel feed ingredient derived from a porcine blood hydrolysate (PBSH), and *in vivo* its bioactivity in a fish feeding trial with gilthead sea bream as a farmed fish model. Such in vivo testing was based on a multi-layer metabolic, transcriptional, and gut microbiota approach, including measurements of behaviour, swimming performance and dynamics of fasting weight loss and weight regain during a fasting/refeeding challenge.

## Materials and Methods

PBSH enzymatic hydrolysate was obtained according to Moreno-Mariscal et al. (2025) and then filtered (<10 kDa) and spray-dried. Antioxidant activity and inhibitory activity against dipeptidyl peptidase IV (DPP-IV), neprilysin (NEP), tumor necrosis α-converting enzyme (TACE) and monoacylglycerol lipase (MGL) enzymes were studied. PBSH was fractionated by RP-HPLC and peptide sequences from the most bioactive fraction were identified by mass spectrometry techniques and later synthetised to confirm their biological activity. For the *in vivo* analysis, juvenile fish of approximately 19 g were fed with control (CTRL) or PBSH-supplemented diets for 10 weeks. At the end of this period, blood, liver, intestine and gut microbiota samples were collected after 24 and 48h of fasting for biochemical, gene expression, histological, and gut microbiota composition analyses. After the feeding trial, the pre-feeding fish behaviour was monitored by video recording. In parallel, swimming performance was assessed to determine basal and maximum metabolic activity rates (BMR, MMR) and critical swimming speed (Ucrit). Finally, a fasting and refeeding challenge of ten and eight days, respectively, was performed to assess the fasting weight loss and weight regain during refeeding.

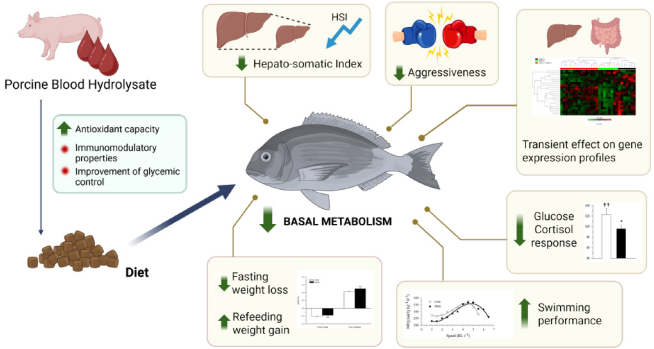


**Figure 1.** Graphical summary representation of the main outcomes (created in https://BioRender.com).

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**Results and Discussion**

As summarized in Figure 1, the optimised hydrolysis of porcine blood resulted in an extract enriched in free amino acids and bioactive peptides with a molecular size lower than 10 kDa. These peptides conferred antioxidant, hypoglycaemic, anti-inflammatory, and immunomodulatory properties to the extract. In fact, five peptide sequences were identified and confirmed as inhibitory of DPP-IV and NEP enzymes. A synergistic effect of these peptides was observed at a concentration of 1mM with inhibition values of these enzymes of 56.43% and 83.54%, respectively. In agreement with these potential benefits, all fish in the trial showed an optimal growth performance (SGR = 2.3; FCR = 1), which was accompanied in PBSH fish by a lower hepatosomatic index and a down-regulated expression of key hepatic lipogenic enzymes (diminished risk of liver steatosis). While no signs of histopathological damage were found in CTRL and PBSH fish, data on gene expression profiling indicated a transient influence of the experimental diet for both liver and intestine profiles, which was prone to reduce overall metabolic rates highlighting an anti-inflammatory appearance in the intestine. PBSH fish also showed an improved welfare condition with lower plasma glucose and cortisol levels in concurrence with a less aggressive behaviour during pre-feeding (30 min before feeding). Otherwise, the PBSH diet enhanced physical performance (20% increase in Ucrit) and reduced basal metabolic rates with a minimised weight loss during starvation, and accelerated weight regain during re-feeding. Finally, the intestinal microbiota was also reshaped by the dietary intervention with a redistribution of dominant bacterial genera associated to the PBSH group.

**Conclusions**

Enzymatic hydrolysis of pig blood offers a sustainable approach to revalorise this by-product by producing a new functional ingredient enriched in essential amino acids and bioactive peptides. The inclusion of the PBSH hydrolysate into fish diets enhances resilience to environmental stressors and improves overall fish welfare and wellbeing contributing to more sustainable and efficient aquaculture practices.

**Funding**

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# SEASONALLY ADJUSTED PROTEIN LEVELS IN ATLANTIC SALMON: A STRATEGY TO ENHANCE WELFARE, IMPROVE FILLET FIRMNESS, AND REDUCE MELANIN SPOTS

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## Introduction

Standard feeding strategies in Atlantic salmon farming are typically size-based, with high-protein diets (>40%) used for juveniles and high-fat diets (≥35%) during grow-out to promote protein sparing (Weihe et al., 2018). Ensuring good fish welfare and fillet quality is essential for sustainable production. Skin wounds are a major welfare concern, increasing vulnerability to infections and contributing to quality downgrading at harvest. Other relevant indicators include normal morphology and eye health (Moldal et al. 2025). Fillet quality is determined by factors such as nutritional composition, appearance, and texture. Visual attributes include the typical salmonid pink-red coloration and the absence of melanized spots, which influence consumer acceptance. Firmness is particularly important for both processing yield and sensory quality (Mørkøre, 2025).

This study evaluated three feeding strategies for autumn-transferred smolts farmed in Arctic conditions, assessing the potential benefits of high-protein diets with high marine protein share applied during winter alone or extended through both winter and spring, with respect to fish welfare and fillet quality.

## Materials and methods

The trial was conducted at the Cermaq farming site Toknebuktneset, Finnmark (70.57°C, 24.07°E, Norway). Each of nine 120 m circumference open sea cages were stocked with 120 000 Atlantic salmon (*Salmo salar* L.) smolts averaging 75 g in June 2021. The trial started in October 2021, when the body weight averaged 570 g. The dietary regimes were:

Control: Standard feed during the production phase, October 2021-October 2022.

HP-W: High protein feed during the winter period from October 2021-March 2022; thereafter the fish were fed the Control feed until harvest.

HP-WS: High protein feed during winter and spring period from October 2021-June 2022; thereafter the fish were fed the Control feed until October 2022,

Potential effects of the dietary treatments on growth performance, morphology, welfare indicators, nutritional status, and sensory quality were assessed at baseline in October 2021 and subsequently in March, July, and October 2022.

## Results and discussion

Over the one-year feeding trial, average body weight increased from 0.57 kg to 3.17 kg. While no statistically significant differences in final body weight were observed among dietary treatments, the control group showed a tendency toward reduced growth, particularly in July and October. The thermal growth coefficient (TGC) averaged 3.23, which is above commonly reported values for salmon farmed under commercial conditions with optimal feeding and environmental parameters. TGC was significantly higher for the HP-W and HP-WS groups compared to the control between March and July. Biological feed conversion ratio (FCR 1.13) and mortality rates (6.9 %) remained acceptable and comparable across all treatments.

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Operational welfare indicators (OWIs), including skin condition and eye lens opacity, indicated improved welfare in fish fed high-protein (HP) diets. In July, wound prevalence was significantly higher in the Control group (26.7%) compared to the HP-WS group (6.7%, $P = 0.04$), and scale loss was greater in the Control group than in the HP-W group ($P = 0.05$). In October, eye lens opacity affected 22.7% of fish in the Control group, versus 4% in both HP groups ($P = 0.03$), further supporting long-term welfare benefits of HP feeding.

Quality grading of whole fish showed a high proportion of superior share (91%), with no significant difference among the dietary regimes. Improvements were observed for fillet integrity and the occurrence of dark melanin spots. At harvesting in October, the share of fillets with severe gaping (score $\geq 3$) and soft texture (breaking force $\leq 6$ N) was halved for the high-protein (HP) groups compared with the Control ($P \leq 0.04$). These results show that feeding the fish HP diets can prevent the development of gaping and soft texture, that are two commercially important traits. Fillet colour, measured using SalmoFan scores, showed that the HP-WS group achieved higher colour score in July (25.2 vs. 23.9; $P < 0.0001$), suggesting an interaction between diet composition and pigment retention. However, at harvesting four months later, visual colour was similar for all dietary groups.

Focal melanized spots are a major quality issue in farmed salmon (Mørkøre, 2025). In March, the prevalence of melanized spots in the cranio-ventral fillet averaged 11.1%, with no significant differences between the groups. By July, the prevalence of the Control group increased (30.7%), while the HP-W and HP-WS groups had lower prevalences (8.3% and 0%, respectively). At harvest, the Control group maintained a prevalence of 20%, while it was lower for the HP-W group (3.5%, $P = 0.05$). No significant difference was observed between the Control and HP-WS group (10%) ($P = 0.22$). From July onward, the number of dark-stained muscle segments was lower in the HP groups, with a significant difference observed between the Control and HP-WS groups in July ($P = 0.05$), and between the Control group and both HP groups in October ($P = 0.02$).

This study is the first to demonstrate that feeding high-protein diets with high marine protein share during the first winter (and following spring) can improve fish welfare by decreasing skin issues and effectively mitigate eye defects and quality issues such as gaping, soft flesh, and melanin deposition at slaughter.

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# ONE FUNCTION, MANY FACES: A MULTI-SPECIES DIVE INTO THE FUNCTIONAL CORE OF FISH GUT MICROBIOMES

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## Introduction

Among the microbiomes closely associated with animals, the intestinal populations represent the most abundant and biodiverse. This microbial association is also characterized by a strong inter and intra-species variability, which however came with a high functional redundancy. These two aspects together make the investigation and the prediction of the microbiota modification an ongoing challenge (Vatsos, 2017). Therefore, the definition of feasible microbial biomarkers and the understanding of the inner bacterial relationships are particularly relevant in the context of aquaculture sector, as it is broadly documented how microbiota can impact the performance and the welfare of farmed animals (Luan et al., 2023). Hence, the aim of the present study was to find similarities and differences in the gut microbial populations of three main European farmed fish (gilthead sea bream, European sea bass, rainbow trout) with focus on the functional role of the core microbiota species using a computational and Bayesian network approach.

## Materials and Methods

The present study was conducted using a total of seven intestinal microbiota datasets, four for European sea bass (*Dicentrarchus labrax*), three for rainbow trout (*Oncorhynchus mykiss*), and an additional meta-analysis sea bream (Sparus aurata) data taken from (Moroni et al., 2025). The considered bacterial profiles have been obtained from different feeding trials focused on the effects of partial substitution of fish meal and fish oil with innovative and alternative feed ingredients. The different microbiota results were used to construct a hierarchical and stochastic Bayesian network (BN) to identify the relationships within the populations and the role of the core microbiota fraction previously identified. Functional characterization of the microbiota was performed using a cluster-based approach, identifying densely connected nodes and inferring their functional contribution using PICRUSt2 protocol (KEGG). Multi-species final comparison was performed using a Partial-Least Square Discriminant Analysis (PLS-DA).

## Results and Discussion

For all the fish species analysed, feed composition and genetic represent the main driving factors, which modulate and change the intestinal microbiota composition. Despite this variability, a reduced number of bacteria, which however represent the most abundant fraction, has been identified as core. In particular, sea bass showed a total of 40 bacteria, while trout only 23. The BN construction allowed the identification of the stochastic structure of the bacterial population for each fish species, and the hierarchical role of core microbiota, highly distributed within the community (Fig. 1A). The importance of this microbial fraction was also confirmed by the inferred functional analysis, as the metabolic profiles of the core-associated clusters played as principal actors in all the pathways identified (Fig. 1B). The results also highlighted that European sea bass and rainbow trout shared similar metabolic routes, but carried out by distinct bacterial communities, confirming that within a strong functional redundancy, the two farmed species presented a completely separate intestinal microbial organization. Furthermore, the importance of the core microbiota as feasible specific signature was also confirmed from an ecological and evolutionary perspective. In fact, the introduction of the gilthead sea bream microbiota profile in the analysis clearly indicated a stronger similarity with European sea bass, confirming that genetic background and environmental factors play a pivotal role in the shaping of the intestinal microbiota (Fig. 1C). These results finally allowed the identification of bacterial markers specific to the two phenotypes, functionally determining and belonging to the core fraction.

## Concluding remarks

This study demonstrates the potential of a functionally core microbiota driven approach to identify bacterial biomarkers that not only reflect taxonomic signatures but also reveal deeper ecological phenotypes. Ultimately, these results provide a valuable tool for monitoring intestinal health status, welfare assessment and defining more sustainable production strategies for numerous aquaculture species.

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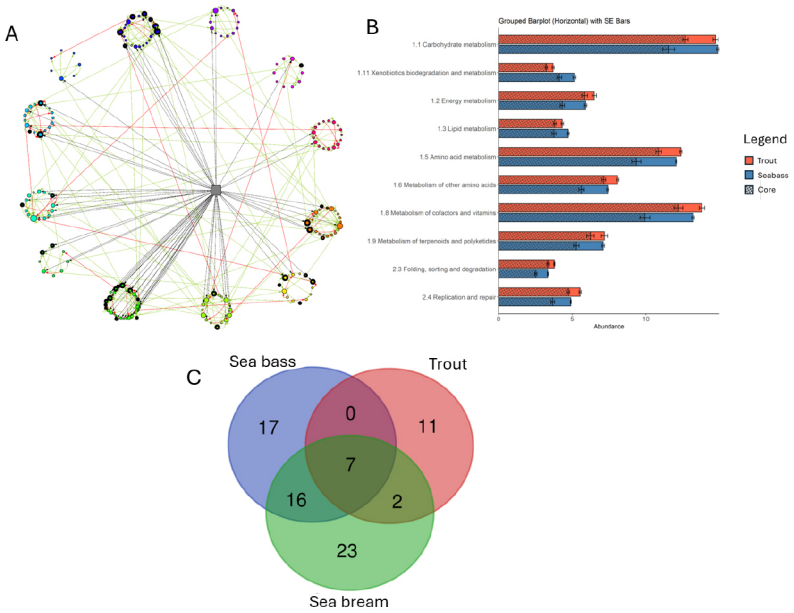


**Figure 1.** A) Bayesian Network and cluster organization. B) Functional analysis of European sea bass and rainbow trout. C) Core microbiota comparison among sea bass, trout, and sea bream.

**Funding**

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# RETHINKING GILTHEA SEA BREAM FARMING WITH SUSTAINABLE DIETS FOR ENHANCED GROWTH AND CLIMATE CHANGE RESILIENCE

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## Introduction

Aquaculture sustainability is increasingly challenged by climate change, with projections indicating more frequent marine heatwaves and prolonged periods of thermal stress (Haberle et al., 2024). While elevated temperatures can enhance growth within species-specific thermal optima (e.g., up to 28°C for gilthead sea bream), exceeding these thresholds disrupts metabolism, impairs immune function, and destabilizes gut homeostasis, resulting in tissue damage and shifts in microbiota composition. Functional dietary additives have shown promise in mitigating these effects; however, the potential of alternative protein sources and novel feed formulations remains unclear (Fernandes et al., 2024). This study evaluated the performance of three successive gilthead sea bream stocks over a four-year temperature profile that mirrors natural climate-driven thermal fluctuations, assessing the long-term effects of alternative protein sources and novel fish feed formulations on growth performance, histopathology, blood metabolites, multi-tissue (liver, white skeletal muscle, anterior intestine, head kidney gene expression) and resilience to environmental stressors.

## Materials and Methods

For the study, gilthead sea bream (*Sparus aurata*) juveniles from Mediterranean origin (Avramar, Burriana, Spain) were raised at the Institute of Aquaculture Torre de la Sal (Spain) under natural photoperiod and temperature conditions. The study spanned three consecutive production cycles (Trial 1: April 2020–December 2021, Trial 2: April 2021–December 2022, Trial 3: May 2022–December 2023). In Trials 1 and 2, attention was focused on growth performance with fish fed standard commercial diets (Biomar, Spain). In trial 3, three experimental diets were tested. The control diet (CTRL) mimicked current commercial formulations with FM, FO and plant ingredients as the main dietary protein and lipid sources. The other two experimental diets were partially or totally devoid of FM with plant ingredients and poultry meal as the main FM replacer in the PAP diet, while the main FM replacer for the named ALT diet were plant proteins together with insect proteins and microbial biomass. Otherwise, salmon-oil by-product and DHA algae oil were used as alternative oils in PAP and ALT diets, respectively. Across the trial, blood and tissue samples were collected in summer, autumn, and winter for biochemical, histological and gene expression analyses, using customized tissue-specific arrays with 106 selected markers of growth, lipid metabolism, antioxidant defence, and immunological status. In parallel, the effect of the nutritional background on stress susceptibility and resilience was assessed in a crowding stress test (changing the density from 16 to 48 kg/m$^3$) by the individual tracking of physical activity and respiratory frequency, using AEFishBIT biosensors externally implanted to the operculum (Calduch-Giner et al., 2022).

## Results and Discussion

The temperature always represents a factor that strongly affects metabolic activity and growth performance of ectotherm animals. Accordingly, the rise of incidence of intense marine heatwaves and heat stress events are worsening risks for aquaculture, especially in the Mediterranean basin. However, the present results exhibited a contrary and promising trend for gilthead sea bream, because the achieved growth align with the historical water temperature records in the Mediterranean Sea along the summers of 2022 and 2023, reaching the commercial size in a 1-2 months shorter period (Fig. 1A). This important finding highlights that a correct feeding schedule is able to promote an enhanced growth with the water temperate rise regardless of the use of different fish feeds. Certainly, the achieved growth with the three tested diets was almost undistinguishable, and no signs of histopathological damage were found in both liver and intestine in any experimental dietary group. However, going deeper with the analyses, the transcriptomic profiles revealed different temporal metabolic strategies that clustered together different fed fish for a given particular tissue. Thus, liver and muscle gene expression patterns clustered together CTRL and ALT groups

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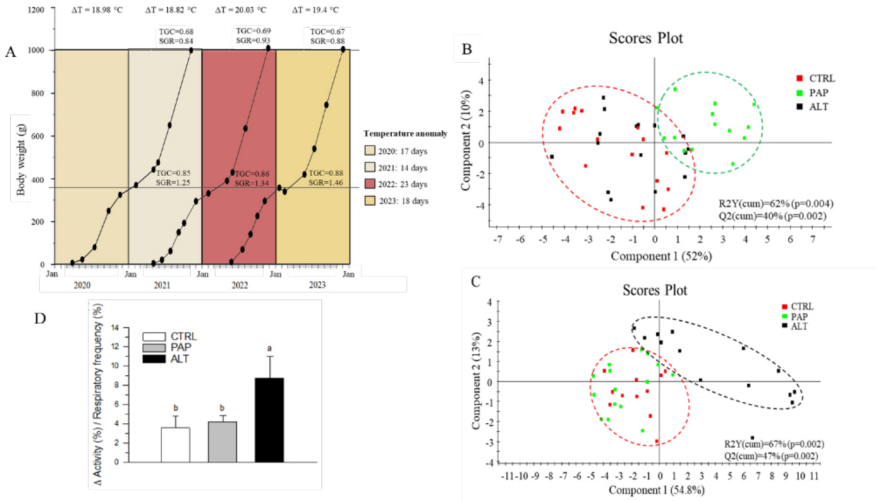


**Figure 1.** A) Growth performance and temperature anomaly during 2020-2023. B-C) Separation of experimental groups in PLS-DA using gene expression profiling in liver and intestine, respectively.

at the end of trial (Fig. 1B), mostly due to the confluent expression pattern of markers of lipid metabolism and mitochondrial respiratory chain. In contrast, the differential expression pattern of immune-related genes of the intestine clustered together CTRL and PAP fed fish (Fig. 1C). These different gene expression features also shaped a different stress response with a more reactive behaviour (increased activity) immediately after stress confinement in ALT fed fish, which rendered an enhanced activity/respiratory quotient with 2-3-fold lower plasma cortisol levels under standard basal conditions (Fig 1D). Accordingly, it was hypothesized that the ALT feed would avoid a chronic stress condition (improved welfare and wellbeing), which supports at the same time a more reactive response when fish are stress challenged.

## Concluding Remarks

This work demonstrates how a correct management of animals during the production cycle, combined with adequate feed formulations, can contribute not only to mitigate the negative impact of climate change, but also to take advantage of a scenario of a global warming. This feature was achieved with different feed formulations, although gene expression analysis and behavioural tests allows different physiological trade-offs that need to be considered in depth to ensure a customized and more efficient aquaculture.

## Funding

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# TOWARDS SUSTAINABLE AQUAFEED SYSTEMS IN THE EU: A COMPREHENSIVE STUDY ON MARKET DYNAMICS, REGULATORY FRAMEWORKS, AND SUSTAINABILITY FACTORS

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**Background & Aims**

The European Commission's 2021 *Strategic Guidelines for Sustainable and Competitive EU Aquaculture* highlight the urgent need to transition towards more sustainable feed systems in aquaculture. In response, the Commission has commissioned a comprehensive study—currently ongoing—focusing on aquafeed systems across the EU, with additional comparative insights from the UK, Norway, and global producers. This study supports policy development by identifying good practices, evaluating sustainability challenges, and proposing data-driven solutions to reduce the sector's dependency on fish meal and fish oil from wild stocks.

The study encompasses five key objectives: (1) mapping the policy and regulatory frameworks and applicable standards for aquafeed; (2) analysing the aquafeed market, including aquafeed products, ingredients and additives, and the respective producers; (3) evaluating traceability and transparency practices across supply chains; (4) exploring trends and readiness levels of emerging and novel feeds, feed ingredients and feed additives; and (5) assessing economic, environmental, and social sustainability dimensions, including the role of certification schemes and circularity in feed production.

**Methods**

The research follows a mixed-methods approach, combining extensive desk-based analysis with field research. The latter includes survey consultations with stakeholders from across the sector including industry, academia, national authorities, producers and farmers, NGOs, amongst others. Emphasis is placed on the various life stages of the predominant commercial species in EU aquaculture including carp, meagre, sole, seabass, seabream, salmon, trout, shrimp, sturgeon, turbot and bluefin tuna.

**Conclusion & Outlook**

The aquafeed, aquafeed ingredient and aquafeed additive sector involves long and complex supply chains, which are threatened further by shifting climate and environmental dynamics, global economic shocks and socio-political threats. The draft results of this study, expected by September 2025 (and anticipated final results by January 2026), will provide a detailed overview of the current state of the aquafeed market, which will inform future guidance and investment in sustainable aquafeed strategies, aligning with EU food security and climate objectives.

# ENVIRONMENTAL BENEFTIS OF POND & WETLAND FISH FARMING AND CHALLENGES AND OPPORTUNITIES IN PROMOTING THOSE BENEFITS

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**Background**

The Strategic guidelines for a more sustainable and competitive EU aquaculture[1] outline the steps required to drive the development of aquaculture in a manner that aligns with goals of the European Green Deal. Low trophic aquaculture, in particular the farming of low-trophic finfish (in ponds, lagoons, estuaries, reservoirs and wetlands) presents a viable option for a resilient, competitive and enviro-compatible aquaculture sector in the EU due to the favourable ecosystem conditions, markets for and historical experience in farming such species. Despite indications that these types of aquaculture can have minimal impact on the environment (and in some cases be beneficial to the environment), there are no defined measures for, and standardized techniques to assess the sustainability and eco-compatibility of these types of aquaculture.

**Methodology & Aims**

The present study aims to assess the environmental benefits of finfish farming in ponds and wetlands through a semi-quantitative analysis of the available scientific evidence and through engagement with key stakeholders. The study seeks to highlight if and how these types of aquaculture can and are being promoted across the Member States, as well as providing recommendations to overcome barriers. The study looks to provide an overview of how these types of aquaculture, and their positive environmental impacts, can be successfully promoted, with the aim that aquaculture in the EU can become a global reference for sustainability.

**Results**

The project highlights the contribution of pond and wetland farming to several ecosystem services across regulating, provisioning and cultural services. Opportunities for developing the sector (including greater recognition and valorisation of ecosystem services, improving climate change resilience and addressing social acceptance challenges) are discussed as potential solutions to challenges facing the sector. Several case studies draw on examples of good practices to illustrate potential pathways for sector development.

This study falls under the EU Commission project *Assessing the environmental benefits of aquaculture and challenges and opportunities in promoting those benefits.*

https://ec.europa.eu/commission/presscorner/detail/en/ip_21_1554

# UNVEILING THE SPECIFIC MICROBIOMES OF BIVALVES: INSIGHTS INTO HOST MICROBIAL DYNAMICS AND PATHOGEN INTERACTIONS IN A SHARED ENVIRONMENT

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**Introduction**

The microbiome is essential for the health and resilience of marine species (Diwan et al., 2023); however, its complexity and host-specific dynamics remain poorly understood. This study presents the first comprehensive analysis of eukaryotic and prokaryotic microbiomes in three ecologically important bivalves - mussels (*Mytilus galloprovincialis*), clams (*Ruditapes philippinarum*), and cockles (*Cerastoderma edule*) - from a shared environment of Meira in the Ría of Vigo (NW Spain), between 2016 and 2018.

**Materials and methods**

The V9 (18S rRNA) and the V4 (16S rRNA) regions genes sequenced using Illumina and Ion Torrent. Alfa diversity, beta diversity (Bray-Curtis, PCoA) and PERMANOVA were used to evaluate eukaryotic and prokaryotic communities for each bivalve sample in different seasons.

Network analyses based on Spearman correlations and corrected CLR data were conducted to detect potential keystone interactions within prokaryote communities of each bivalve and its surrounding environment (mussel-water column, clam-sediment and cockle-sediment). Finally, functional activities associated with keystone taxa from each bivalve-habitat network were also identified using PICRUSt2.

**Results and discussion**

Each bivalve species harbored a distinct, host-specific microbiome with stable composition across seasons pointing that intrinsic host factors affect the composition of the bivalve microbiome regardless of environmental conditions and time (Neu et al., 2021).

Specific parasites, including *Mytilicola intestinalis* in mussels and trematodes such as *Bucephalus minimus* in cockles, were identified. *Perkinsus olseni* and *Marteilia cochilia,* protozoans linked to bivalve mortality and ecosystem disruption under environmental stress, were also detected.

Endozoicomonas and Vibrio dominated prokaryotic communities. A meta-analysis showed that despite the microbial diversity in the water column and sediment, each bivalve maintained its own stable and specific microbiome, distinct from its environment. These results suggest that bivalves can regulate their core microbiome through host-selective mechanisms (Brennan & Gilmore, 2018; Pierce & Ward, 2018)

We could identify *Vibrio*, *Woeseia* and *Lutimonas* as keystone genera shaping these microbiomes through competitive and cooperative interactions (Lu et al., 2022; Zelezniak et al., 2015). Mutualistic relationships enhanced host health via metabolic and defensive roles involving the biosynthesis of secondary metabolites.

**Conclusions**

Understanding host-microbe-environment interactions, particularly concerning pathogens, underscores the importance of improving ecosystem resilience and sustainability. These findings provide essential insights into microbiome dynamics, offering valuable perspectives for ecosystem and bivalve health monitoring and management.

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# MANIPULATION EFFECTS OF JACK MACKEREL MEAL IN THE DIET SUBSTITUTING 50% OF FISH MEAL WITH CORN PROTEIN CONCENTRATE ON THE GROWTH PERFORMANCE AND FEED AVAILABILITY OF OLIVE FLOUNDER (*Paralichthys olivaceus*)

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## Introduction

Fish meal (FM) is expensive because of its increasing demand and unstable production worldwide. Therefore, searching for an alternative protein source for FM is necessary for sustainable aquaculture. Nevertheless, low-FM diets often lead to deteriorated palatability and reduced feed consumption by fish, ultimately resulting in lowered growth performance of fish. Therefore, this study aims to investigate manipulation effects of jack mackerel meal (JMM) in a diet substituting 50% of FM with corn protein concentrate (CPC) on the growth performance and feed availability of olive flounder (*P. olivaceus*).

## Materials and Methods

Seven experimental diets were prepared to be isoproteic (52.0%) and isolipidic (12.0%) (Table 1). The control (Con) diet contained 60% FM. In the Con diet, 50% of FM was substituted with CPC and then graded (0%, 10%, 20%, 30%, 40%, and 50%) levels of JMM were included at the expense of FM, referred to the CPJ0, CPJ10, CPJ20, CPJ30, CPJ40, and CPJ50, respectively. A total of 525 juvenile (6.3 ± 0.02 g; mean ± SE) olive flounder was randomly distributed into 21 of 50-L flow-through tank (25 fish/tank) with triplicate. Fish were hand-fed to apparent satiation twice a day for 50 days.

## Results

Weight gain (WG) of olive flounder fed the Con and CPJ50 diets was significantly ($P < 0.003$) higher than that of fish fed the CPJ0 diet (Figure 1). Specific growth rate (SGR) of fish fed the Con diet was significantly ($P < 0.001$) higher than that of fish fed the CPJ0, CPJ10, CPJ20, and CPJ30 diets. In orthogonal polynomial contrast, WG and SGR of fish revealed linear ($P = 0.0009$ and $P = 0.0006$, respectively) relationships with dietary inclusion levels of JMM. Regression analysis showed linear relationships as the best suitable models between increased levels of JMM in diets substituting 50% of FM with CPC and WG ($Y = 0.597540X + 17.9022$, $P < 0.0003$, $R^2 = 0.5851$) and SGR ($Y = 0.000437X + 0.0270$, $P < 0.0003$, $R^2 = 0.5850$), respectively. However, feed consumption and feed utilization were unaffected by dietary treatments.

## Conclusion

Incorporation of 10 and 40% JMM in the low-FM diets substituting 50% of FM with CPC could achieve comparable WG and SGR of olive flounder, respectively, to fish fed the 60% FM based diet.

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Index mundi, 2025. Fish meal production by country in 100 MT.
Index mundi, 2025. Fish meal monthly price.

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Table 1. Ingredients and proximate composition of the experimental diets (%, DM basis).

| | Experimental diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | Con | CPJ0 | CPJ10 | CPJ20 | CPJ30 | CPJ40 | CPJ50 |
| Fish meal | 60.0 | 30.0 | 24.0 | 18.0 | 12.0 | 6.0 | |
| Corn protein concentrate | | 25.7 | 25.7 | 25.7 | 25.7 | 25.7 | 25.7 |
| Jack mackerel meal | | | 6.0 | 12.0 | 18.0 | 24.0 | 30.0 |
| Fermented soybean meal | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 |
| Wheat flour | 15.0 | 17.9 | 18.0 | 18.0 | 18.1 | 18.2 | 18.3 |
| Fish oil | 4.0 | 5.4 | 5.3 | 5.3 | 5.2 | 5.1 | 5.0 |
| Soybean oil | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Choline | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Crude protein | 52.3 | 52.4 | 52.1 | 52.1 | 52.4 | 52.2 | 52.4 |
| Crude lipid | 10.3 | 7.0 | 7.5 | 7.4 | 7.5 | 7.3 | 7.3 |

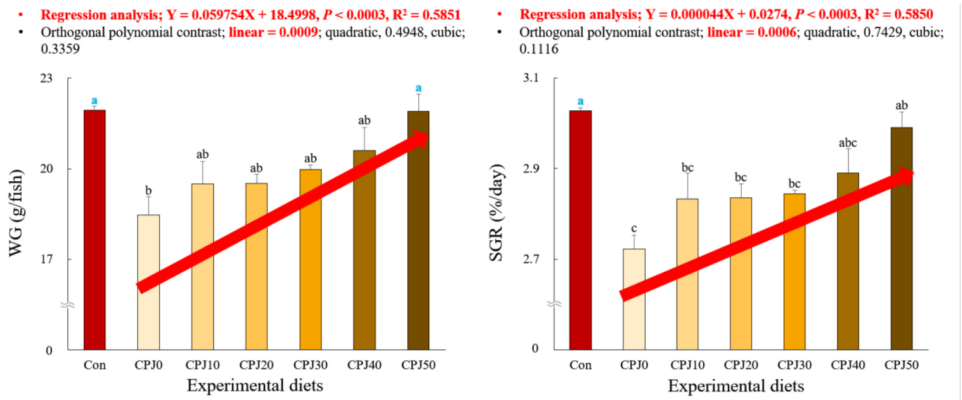


Figure 1. Weight gain (WG, g/fish) and specific growth rate (SGR, %/day) of olive flounder fed the experimental diets substituting 50% of fish meal with corn protein concentrate with the inclusion of graded levels of jack mackerel meal (means of triplicate ± SE) (***P* < 0.003** and ***P* < 0.001**, respectively).

# EFFECT Of THYROID HORMONE ADMINISTRATION ON THE GROWTH Of *Epinephelus malabaricus*

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In addition to growth hormone (GH) synthesized in the pituitary gland, various hormones and peptides are involved in the control of growth performance in fish. However, it remains unknown how the fish growth is regulated comprehensively by the endocrine networks, although understanding the endocrine system is important to maximize and optimize the target species in aquaculture. The present study aimed to explore the effect of thyroid hormone (TH) and thiourea (TU), an antithyroid agent, on the growth of the Malabar grouper, *Epinephelus malabaricus*, which is an important species in aquaculture in southern Japan.

Juveniles of the Malabar grouper (142.1 ± 3.28 mm) used in this study were obtained from Okinawa fish framing center and housed in tanks with seawater at the Nakagusuku Aquaculture Innovation Center. Fish were fed commercial pellets with thyroxine (T4, T4 group), TU (TU group), and T4 and TU (T4+TU group), and without T4 and TU (control group). Fish were reared with feeding of these diets for 4 weeks, and body weight (BW), condition factor (CF), specific growth rate (SGR), feeding efficiency (FE), and hepato-somatic index (HSI) were measured. Total RNA was extracted from the liver and pituitary gland, and cDNA was obtained by reverse transcription. The transcript levels of insulin-like growth factor-1 (*Igf-1*) in the liver and thyroid stimulating hormone (*TSH*) and growth hormone (*GH*) in the pituitary gland were measured by quantitative PCR.

BW, SGR (Fig. 1), FE, and HSI of the control and T4 groups were significantly higher than those of the TU group, suggesting that TH is involved in the growth of juvenile Malabar grouper. The transcript levels of *Igf-1* increased in the TU group. Although the transcript levels of *GH* were not significantly different among groups, those of *TSH* significantly increased in the T4+TU and TU groups. These results indicate that the hormone network in relation to TH synthesis and secretion is involved in the growth of this species through the expression alternation of growth-related genes in the liver. This study was supported in part by COI-NEXT (JPMJPF2012) of JST program.

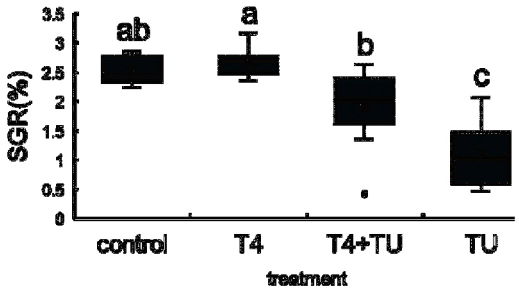


Fig. 1. Effect of hormone treatment on specific growth rate (SGR) of the Malabar grouper. Control; treatment without hormones, T4; single treatment with thyroxine, T4+TU; co-treatment with thyroxine and thiourea, TU; single treatment with thiourea. Different letters show statistic difference ($P < 0.05$).

# INFLUENCE OF MICROALGAE-ENRICHED DIET ON SPERM QUALITY PARAMETERS IN COMMON CARP *Cyprinus carpio*

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## Introduction

Aquaculture is a key contributor to global food security. However, it faces numerous challenges that threaten its sustainability and its ability to meet the growing demand for animal protein (Ruben et al., 2025). The common carp is one of the most important freshwater species both in Europe and globally (FAO, 2024). Meeting the nutritional requirements of broodstock is a key factor in ensuring a healthy breeding population, successful reproduction, and ultimately, healthy offspring (Fernández-Palacios et al., 2011). Alternative feed additives, such as microalgae (Ansari et al., 2021), may improve gamete quality and contribute to more consistent reproductive outcomes. However, data on reproductive parameters of broodstock fed algae-supplemented diets remain limited. The aim of our study was to investigate the effects of 3% microalgae feed supplement on sperm quality and quantity in common carp.

## Materials and methods

The experiments were conducted at a Hungarian fish farm (Szegedfish Ltd). To carry out the sperm quality and quantity analyses, two groups were established. The control group was fed a conventional diet, while the experimental broodstock received feed enriched with 3% microalgae (*Chlorella* spp., *Scenedesmus* spp., *Coelastrella* spp., *Acutodesmus* spp.) 1 month prior to the spawning season. Sampling was performed at three consecutive date ($N = 6$ individuals per sampling and group) during the spawning season. Average body weight and length were recorded prior to stripping. During sampling, the following parameters were measured and calculated: total volume of milt (TVM), volume of milt per kg of body weight (VOM), spermatozoa per millilitre (SPZ), total sperm production (TSP), total sperm production per kilogram of body weight (TNS), osmolality and pH of the seminal plasma, as well as sperm motility and kinetic parameters (Cejko et al., 2011). Sugar and ion content: glucose (GLU), fructose (FRU), calcium (Ca), potassium (K), sodium (Na), and magnesium (Mg), and sperm fatty acid profile: total fatty acids (TFA), saturated fatty acids (SFA), unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (OM3), and omega-6 fatty acids (OM6) were also assessed.

## Results

Results were evaluated using two-way ANOVA. First, control and algae groups were compared at the same temperature. Second, values at different water temperatures (18,5 °C; 20,5 °C; 21,8 °C) were compared within each group.

### Traditional Sperm Parameters

At 20.5 °C, VOM and TNS were significantly higher in the algae group. OSM differed significantly across all sampling times. At 21.8 °C, pH was lower in the control group.
In the control group, SPZ increased with temperature, OSM peaked at 20.5 °C, and pH was highest at 18.5 °C. BCF was highest at 18.5 °C. In the algae group, SPZ, TSP, and TNS peaked at 20.5 °C, while pH was highest at 18.5 °C.

### Sugar and Ion Content

At 20.5 °C, K and Mg were higher in the control group; at 21.8 °C, K was higher in the algae group. In the control group Ca, K, Na, and Mg concentrations peaked at 20.5 °C. In the algae group, K concentration was significantly lower at 18.5 °C, while Na peaked at 20.5 °C. Mg concentration also differed significantly between 18.5 °C and 20.5 °C.

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Fatty Acid Content

Fatty acid composition showed the largest differences. At 20.5 °C, TFA was higher in the control group; at 21.8 °C, it was higher in the algae group. Fatty acid composition showed the largest differences. At 20.5 °C, TFA was higher in the control group; at 21.8 °C, it was higher in the algae group. In the control group, all fatty acid parameters (except OM6) peaked at 20.5 °C. In the algae group, most values were significantly higher at 20.5 °C than at 18.5 °C. No significant differences were observed in this group between the lowest and highest temperature.

**Discussion and conclusion**

A 3% algae supplementation significantly improved sperm quality parameters compared to the control. Physiological values (sugar, ion, fatty acids) were influenced by both treatment and temperature, with highest values generally at 20.5 °C. The algae group showed increasing quantitative values with rising temperature.

Our findings suggest that pre-spawning algae supplementation improves sperm quality in common carp under optimal temperature. Future research should include closed-system, multi-group experiments, extending to fertilization trials.

**Acknowledgements**

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# SEQUENTIAL INTEGRATED MULTI-TROPHIC AQUACULTURE IN SALT MARSHES: TROPHIC RELATIONSHIP BETWEEN RAINBOW TROUT, OYSTER, CLAM AND SHRIMP

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## Introduction

Integrated Multi-Trophic Aquaculture (IMTA) is one of the most promising pathways toward sustainable aquaculture systems. Its goal is to reduce negative environmental impacts while increasing productivity. It is a nature-based solution that is conceived to mimic natural ecosystems by combining multiple species from different trophic levels into a single system. The inorganic and organic waste produced by fed aquaculture species (e.g., fish) is consumed and recycled by secondary autotrophic species (e.g., phytoplankton, macroalgae) and/or heterotrophic species (e.g., filter feeders, detritivores). In this way, the by-products of one species become resources for another. However, in marine environments, commercially scaled IMTA systems remain rare due to their management complexity and the lack of knowledge about the role of each productive component. It is therefore essential to develop solid scientific understanding of each species' role and knowledge about their interaction in nutrient and energy use.

Oyster farmers in the salt marshes of Nouvelle-Aquitaine (France) already practice a summer polyculture system that combines farming of kuruma shrimp (*Marsupenaeus japonicus*) with oysters (*Magallana gigas*), using supplemental exogenous feed. A strong demand from the industry aims to further diversify aquaculture practices by introducing winter farming of rainbow trout (*Oncorhynchus mykiss*) into this system. The main objective of this study was to assess the extent to which (1) trout farming could increase the productivity of oysters and clams (*Ruditapes philippinarum*) when reared together in the same ponds during winter and (2) summer production of shrimp, oyster and clam could be supported by the nutrients that accumulated during winter**.** We hypothesized that semi-intensive winter production of rainbow trout, fed exogenous feed, would cause organic nutrients to accumulate in the sediment which would increase the development of benthic invertebrates in the sediment (e.g., benthic macroinvertebrates), as well as phytoplankton and zooplankton in the water column. Benthic invertebrates could serve as a food source for shrimp, while phytoplankton and zooplankton are the primary food sources for oysters and clams.

## Materials and Methods

The experiment was performed in six salt marsh ponds of ca. 600 m² each at the Lycée de la Mer et du Littoral in Bourcefranc-le-Chapus (France). During the winter phase (November 2024 to March 2025), in three of the ponds, oyster and clam were reared with rainbow trout fed commercial feed pellets *ad libitum* (IMTA treatment). In the three other ponds, oyster and clam were reared without rainbow trout (no treatment (i.e., control)). At the end of the winter phase, survival and growth rates of clam, oyster and trout; organic carbon, nitrogen and phosphorus contents of sediment; and phytoplankton, zooplankton and benthic macroinvertebrate productivities were measured in each pond.

The food sources used by rainbow trout, clam and oyster were determined using stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Five individuals of each species were collected in each pond. Sediment organic matter (SOM) and suspended organic matter (SPOM) were also sampled in each pond. Sampled were dried, weighed and packed into pressed tin capsules for simultaneous $\delta^{13}C$ and $\delta^{15}N$ analysis, using an isotope ratio mass spectrometer interfaced with an elemental analyzer. The contribution of food sources to diets were estimated using Bayesian stable isotope mixing models.

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**Results and discussion**

During the winter phase, sediment in both treatments had similar organic carbon, nitrogen and phosphorus contents.

The $\delta^{13}C$ and $\delta^{15}N$ values of rainbow trout indicated that the fish consumed only the commercial feed and not the natural food sources in the ponds, such as benthic macro invertebrates. This result was expected, since the fish had been fed a highly palatable exogenous feed *ad libitum*.

Clam and oyster had the same survival and growth rates, as well as similar $\delta^{13}C$ and $\delta^{15}N$ values, in both treatments. Mixing models indicated that the oyster consumed both SPOM and SOM. Clam were $^{13}C$-depleted and $^{15}N$-enriched compared to oyster. The isotopic niches of oyster and clam did not overlap, which suggests that they are complementary filter-feeder species in IMTA ponds. Clam had similar $\delta^{13}C$ values in both treatments but significantly lower $\delta^{15}N$ values in the IMTA treatment than in the control, likely due to having consumed fish-feed waste. The commercial feed, which contained plant ingredients, had lower $\delta^{15}N$ values than the natural food sources in the ponds did.

In the IMTA treatment, the lack of increase in organic carbon, nitrogen and phosphorus contents in the sediment was likely due to fish biomass not exceeding the assimilation capacity of the ponds, or perhaps due to clam consuming fish waste effectively, which decreased biodeposition in the IMTA ponds.

The summer phase of the experiment will end in October 2025. Its results will help understand the extent to which nutrients accumulated with or without fish in winter support the production of oyster, clam and shrimp in summer.

# ENVIRONMENTAL SUSTAINABILITY OF LUXURY AQUACULTURE PRODUCTS UNDER PRESENT AND FUTURE CLIMATE SCENARIOS

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## Introduction

Today's luxury food products are increasingly expected to meet high environmental sustainability standards. New generations of consumers no longer seek only sensory excellence and exclusivity but also demand ethical and ecological values (Li & Kallas, 2021; Menozzi et al., 2020). As a result, sustainability has become a defining feature of modern luxury, where quality can be measured not only by taste and rarity, but also by the product's environmental impact. While aquaculture remains a vital sector for providing affordable protein to the human growing population, it also includes luxury products, such as caviar and bottarga, or traditionally associated with exclusivity but now becoming increasingly accessible thanks to advancements and expansion in aquaculture practices, such as oysters. While many studies have assessed the environmental impact of farmed seafood, such as salmon, mussel, or tilapia (Gephart, J. A., et al., 2021), only a few focused-on luxury products (Dias et al., 2025; Melas et al., 2024; Napolitano et al., 2025; Vélez-Henao, J. A., et al., 2021). The aim of this research is to quantify the environmental impact of caviar and oyster production systems, incorporating a wide range of case studies to reflect the variability associated with farming methods and environmental conditions. Furthermore, the study aims to develop and analyze alternative scenarios, within the framework of climate change, to provide evidence-based recommendations for producers to uphold high sustainability standards.

## Materials and methods

This research is part of the PhD project "Environmental sustainability of luxury aquaculture products under present and future climate scenarios" developed in collaboration with "CREA" an Italian research institute and the University of Stirling (UK). Environmental impacts associated to luxury aquaculture productions, will be analyzed through the Life Cycle Assessment (LCA), a standardized method widely used. In this study, the environmental performance of the various products will be compared using different functional units (FU), including mass units (e.g., 1 kg), economic (e.g., 100 €) or new specific indicators that can better fit these specific products (e.g., sensory score, aromatic/taste profiles, VOC's quantity, etc..). Being luxury food products not merely intended for nourishment having functions with social and cultural implications, since they are not seen as part of a healthy diet, but rather as "unnecessary pleasure" it is complicated to find a dimension in which to measure them. Finally, a simulation modelling of the effects of environmental variables, expected to change in the next years due to climate change, on aquaculture luxury productions will be carried out in different geographical locations. The results of simulations will be matched with the direct experience of the farmers and the effects observed during recent extreme weather events, causing higher mortalities and the need for adaptation strategies. This information will be used to conduct scenario analyses to assess the effects of climate change and short- and long-term adaptation strategies on the environmental performance of the production chains.

## Results

Preliminary results on the Italian caviar supply chain show a relatively high environmental impact with high variability depending on the species (e.g. Global Warming (GW) potential ranged between 53.4 and 82.9 kg CO2 eq/kg of caviar), mainly due to the long production cycle and related high feed use (Napolitano et al., 2025). Impact levels are also influenced by how they are allocated between co-products (caviar and meat). Scenario analysis suggests that early female detection through genetic techniques could improve resource efficiency. For oysters, environmental performance appears generally good across sites ranging between 1.85 – 2.5 (kg CO2 eq/kg of oysters). Differences in cycle length and product traits, such as taste and aroma, may significantly influence results and pose methodological challenges in LCA integration.

## Conclusions

This work will give an overview on environmental performance of different luxury aquaculture products, considering factors such as different farming methods, environmental variables, co-product allocation, intensification scenarios and climate change.

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# INTEGRATING PRECISION AQUACULTURE STRATEGIES WITH SUBMERSIBLE PEN TECHNOLOGIES

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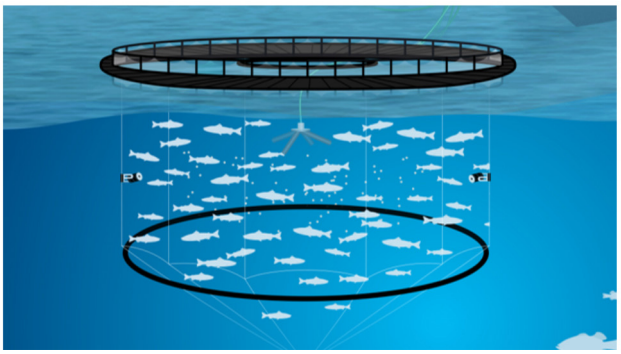
Warm water aquaculture is expanding into open ocean areas, necessitating the submersion of aquaculture systems to avoid surface energy and warming temperatures. Specialized equipment is required to house fish, facilitate their growth, and monitor growing conditions. An integrated system of equipment and technology has been developed to address these needs. Submersible net pens can stay submerged throughout most of the grow-out cycle, allowing for a majority of the activities, from feeding to mortality removal, to be accomplished while submerged and in environments that the fish are most comfortable with. Waterborne feeding also offers many advantages, including a significant reduction in energy use and improved feed conversion ratios (FCR) due to feed pellets staying more intact. Stocking and harvesting, however, need to be done at the surface but can be scheduled around weather conditions.

Precision aquaculture is essential for the success of this system. Wireless sensors distributed throughout the site constantly measure environmental parameters such as salinity, temperature, dissolved oxygen, and other necessary data that can be viewed in real time. High-definition cameras connected to an efficient data transmission backbone allow feed managers, assisted by cutting-edge machine learning and AI algorithms, to optimize feeding regimes. Environmental data, fish behavioral data during feeding, and pellet detection provide detailed guidance for efficient feed delivery and consumption.

In addition to optimizing feeding, biomass estimations are collected and analyzed to help prescribe upcoming feeding levels and amounts and advise other managers in the company when the fish will be ready for harvest and even how much to expect. Furthermore, purchasing managers can monitor feed consumption and be ready to place orders to achieve just-in-time inventory for feed.

This fully integrated approach ensures efficient and sustainable aquaculture practices, enhanced productivity, and reduced environmental impact.



# ADVANTAGES AND CHALLENGES OF INCORPORATING RAS INTO MARINE HATCHERIES

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As opposed to both nursery systems and ongrowing RAS units, marine hatchery systems have multiple inputs that pose unique challenges to RAS units, but once addressed also offer substantial advantages. The main challenges are addressing the cycling of live feeds, flushing and removing them from the system, while the advantages are focused on a stable larval rearing environment and reduced energy costs for enhanced temperature control critical during the larval phases for some marine species.



RAS Hatchery at the University of Miami

# SERUM-FREE ADAPTATION OF FISH CELL LINES FOR MORE SUSTAINABLE AND ETHICAL *IN VITRO* RESEARCH

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## Introduction

There is an ongoing effort in the scientific community to minimize the use of animals in research. *In vitro* studies have been a major development toward achieving this goal. However, even in *in vitro* methodologies, animal-derived products are still used. In cell culture, fetal bovine serum (FBS) is widely used as a supplement in media. FBS purpose in cell culture media is to provide essential amino acids, nutrients, growth factors, and hormones, all of them necessary for the cell to growth, proliferate, and differentiate.

However, FBS is not chemically defined which results in a high degree of variability between batches. This can affect experimental results and their reproducibility, as well as introduce unknown molecules and contaminants that could interfere with certain analyses (Chung et al., 2025). Furthermore, from an ethical point of view, the use of FBS in research raises concerns regarding animal welfare. Ensuring animal welfare is essential at all levels, both in aquaculture practices and in aquaculture research. Likewise, for aquaculture to become truly sustainable, the research supporting it must also adopt more sustainable and ethical approaches. Despite all the inconvenience, FBS continues to be used since no suitable alternative has yet been found. The main objective of this work is to contribute to the establishment of cell lines capable of growing in serum free media (SFM).

## Materials and methods

For this study, *Epitheloma papulosum cyprinid* (EPC) cell line from fish and A549 and Huh7 cell lines from human were used. Five commercial media were tested: basal medium normally used with the cell line (m0), OptiMEM (m1), MegaCell DMEM (m2), MegaCell RPMI (m3), ExCell 293 (m4), and DMEM/F12 PLUS (m5).

The approach consisted of a semi-indirect adaptation. The adaptation was direct to 2% FBS and then was gradually reduced through several steps from 2% to 0%. Passages to a lower concentration were performed when cells reached 90% confluence, using a 1:3 split ratio. Total number of cells, growth rate, cell size and viability by Trypan Blue staining were determined. After three consecutive passages at 0% FBS, the adaptation process was considered complete, and the cell lines were then cryopreserved.

## Results

All three cell lines (EPC, A549, and Huh7) were successfully adapted to SFM. Both human cell lines were adapted to m5, meanwhile the fish EPC line was adapted to m4. The adaptation process required 6-7 passages in all cases, but the total duration varied. A549 and Huh7 completed the adaptation in 28 and 22, respectively, whereas EPC required 111 days.

Although passages were always carried out when the cells reached 90% confluence, the total number of cells varied significantly throughout the adaptation process. In EPC, the cell count returned to baseline levels by the end of the adaptation. However, A549 and Huh7 maintained lower cell count at 0% FBS. This reduction was not due to increased cell death, as cell viability remained relatively stable throughout all passages in all three lines, with cell death rates consistently around 3-4%. Instead, the lower cell count was associated with an increase in cell size. Fewer cells were needed to reach the same confluence due to the larger volume occupied by each cell.

In terms of growth rate, cells proliferated rapidly during the initial passages, requiring subculturing every 24 hours. However, as FBS concentration was gradually reduced, the time between passages increased. By the final passage at 0% FBS, the time between passages had extended to 3 days for Huh7, 10 days for A549, and up to 24 days for EPC.

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## Discussion

This study proves the possibility of adapting cell lines to grow in SFM, which represents a great advance towards more ethical and reproducible methodologies. The results show a significant difference between the total duration of the adaptation process between human and fish cell lines, which could be attributed to physiological differences such as varying sensitivity to growth factor (Jozef et al., 2025; Radošević et al., 2016). Additionally, each cell line adapted to a different medium: EPC was successfully adapted to m4, while both human cell lines thrived in m5. This is likely due to differences in their specific requirements for amino acids and other nutrients (Jozef et al., 2025; Liu et al., 2017).

The variation in the total number of cells is explained by the increase in cell size, resulting in fewer cells occupying the same surface area at confluence. This may be due to the fact that, under starving conditions, cells activate mechanisms such as apoptosis. Another possible explanation is cell cycle arrest in G1 phase, where cell growth occurs. Further studies may be needed to explore the physiological and molecular differences between adapted and non-adapted cell lines.

## Conclusion

The use of chemically defined medium enhances experimental reproducibility and facilitates cross-study comparisons. The successfully adapted cell lines provide a foundation for future research and support the 3Rs principles in science: replacement, reduction, and refinement. Moreover, implementing serum-free systems in aquaculture research contributes to making the field more sustainable and ethically aligned. These advances are essential to ensure that aquaculture development is supported by responsible scientific practices.

## Funding

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# DEVELOPMENT OF LIMPET REPRODUCTION: SPAWNING INDUCTION IN *Patella aspera* AND *P. gomesii* FROM AZORES

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## Introduction

Spawning induction in patellid limpets is essential for aquaculture and conservation, as these broadcast spawners rely on synchronized gamete release for reproduction (De Girolamo *et al.*, 2024). Published guidelines favour non-invasive environmental cues, particularly thermal shock and intense aeration, which successfully stimulate spawning in species such as *Patella aspera*, *P. candei*, *P. caerulea*, and *P. ferruginea* (Castejón *et al.*, 2022; Nunes *et al.*, 2024). Pre-conditioning of broodstock and timing induction to natural reproductive cycles improve success (Castejón *et al.*, 2022; De Girolamo *et al.*, 2024). Such methodological developments represent significant progress toward the establishment of closed-cycle limpet aquaculture, thereby enhancing the potential for sustainable resource exploitation and contributing to the restoration of natural populations (Castejón *et al.*, 2022). Nevertheless, critical gaps persist in spawning induction reliability. The present work directly addresses new induction trials advancing standardized practices for reproducible gamete production in Azorean limpet species.

## Material and methods

Two spawning induction series (2 trials + 1 trial) were conducted for each species, *Patella aspera* and *P. gomesii*, during the reproductive season, adapting methodologies described by Castejón *et al.* (2022) and De Girolamo *et al.* (2024). Each trial comprised 40 individuals, distributed equally among four treatments (10 individuals per treatment). Induction treatments included: thermal shock (high and low temperature), intense aeration, gamete addition, osmotic shock, and a combined treatment (low temperature + aeration + gamete addition + osmotic shock). Prior to treatment, a one-hour pre-treatment under dry, room-temperature conditions was applied. Subsequently, each specimen was placed in an experimental vessel containing 700 mL of sterile filtered seawater (FSW) for a 4.5 h treatment exposure period. At the end of the treatment, FSW was totally renewed (400 mL at environmental temperature). Gamete emission was monitored continuously during treatment and every 30 minutes for 3 hours during the post-treatment. Water temperature, dissolved oxygen, and salinity were recorded throughout all phases. Finally, at least 45% of the limpets from each spawning induction series were dissected to calculate the gonadosomatic index (GSI) (spawners excluded), and gametes released were quantified for comparative analysis.

## Results

In addition to the influence of gonadal maturation, as indicated by the gonadosomatic index (GSI), preliminary results demonstrated that the type of induction treatment was a key determinant of spawning success in both species. The combined treatment yielded the highest efficacy for male gamete release, whereas no oocyte emission was observed in females under any treatment. Sperm release occurred predominantly during the post-treatment phase (Table 1).

## Discussion

Overall, spawning induction was successfully reached in both species, with the combined treatment demonstrating the greatest efficacy, even in cases where GSI values were higher for alternative treatments. Spermatozoa concentrations were variable, with maximal values typically observed in the post-treatment phase. These findings contribute to advancing the understanding of spawning dynamics in Azorean limpets. However, further investigation is required, particularly regarding gamete quality, consistency of spawning responses and scalability.

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**Table 1:** Spawning induction experiments with *P. gomesii* and *P.aspera*. Series 1, trial 1 is represented by treatments: thermal shock – high and low temperature – and intense aeration; series 1, trial 2: gamete addition, osmotic shock and combined; series 2, trial 1: intense aeration, osmotic shock and combined. Nd, number of dissected specimens; GSI, mean gonadosomatic index (%); T, treatment (CB, combined treatment; OS, osmotic shock; IA, intense aeration); Ns, number of spawners; SC DT, released – during treatment – spermatozoa concentration (cells/mL); SC PT, released – post-treatment – spermatozoa concentration (cells/mL).

| Date | Species | Series | Trial | Nd | GSI | T | Ns | SC DT | SC PT |
|---|---|---|---|---|---|---|---|---|---|
| 26/03/2025 | *P. gomesii* | 1 | 1 | 20 | 9.29 | CB<br>CB | 1<br>2 | 1.8 × $10^5$<br>N/A | 3.60 × $10^4$<br>3.12 × $10^5$ |
| 01/04/2025 | *P. gomesii* | 1 | 2 | 30 | 8.68 | CB<br>CB<br>CB<br>OS | 3<br>4<br>5<br>6 | 2.38 × $10^5$<br>6.00 × $10^3$<br>1.4 × $10^5$<br>N/A | N/A<br>4.60 × $10^6$<br>N/A<br>1.60 × $10^6$ |
| 23/05/2025 | *P. gomesii* | 2 | 1 | 20 | 9.30 | | | | |
| 08/04/2025 | *P. aspera* | 1 | 1 | 20 | 7.88 | IA<br>CB | 1<br>2 | 5.77 × $10^5$<br>1.76 × $10^5$ | 1.73 × $10^4$<br>2.28 × $10^6$ |
| 14/04/2025 | *P. aspera* | 1 | 2 | 20 | 7.48 | CB<br>CB | 3<br>4 | 2.96 × $10^5$<br>N/A | 3.44 × $10^5$<br>1.60 × $10^5$ |
| 20/05/2025 | *P. aspera* | 2 | 1 | 20 | 3.60 | | | | |

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# INTERACTION BETWEEN DIFFERENT DIETARY LIPID CONTENT AND REARING AT TWO DIFFERENT TEMPERATURES IN FLATHEAD GREY MULLET *Mugil cephalus*

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## Introduction

Flathead grey mullet (*Mugil cephalus*) is a catadromous, omnivorous fish species that has recently gained attention from researchers due to its potential as a sustainable, low-trophic-level candidate for aquaculture. Its omnivorous feeding habits and resilience to high temperatures make it particularly suitable in the context of climate change. For these reasons, the mullet represents an interesting model species for studying the interactions between environmental factors, such as temperature, growth performance, and feeding strategies. Understanding the effects of temperature is especially relevant in the current scenario of global warming. Optimizing feeding strategies in relation to environmental conditions is essential to improving aquaculture efficiency and sustainability. Among abiotic factors, temperature stands out as a key driver, significantly influencing metabolism, nutrient utilization, animal welfare, and gut health.

## Materials and Methods

Two isoproteic experimental diets were formulated to contain two lipid levels (Diet 10% and Diet 15%) and were tested at two temperatures: 22 °C and 28 °C. Diets were administered to triplicate groups of 30 fish each (initial weight: 90.5 g) over a period of 100 days at both temperatures. During the trial, feed was provided to apparent satiation during a single 6 hour meal per day, and dissolved oxygen was maintained above 7.5 mg $L^{-1}$. At the end of the trial, final body weight (FBW), specific growth rate (SGR), feed intake (FI), and feed conversion ratio (FCR) were calculated. Samples for gut evacuation analysis were collected at 1, 3, 5, 7, 9, and 12 hours post-feeding, while samples for microbiota analysis were taken at 4 hours post-feeding. Differences among treatments and temperatures were considered significant at $p < 0.05$ (two-way ANOVA followed by Tukey's test).

## Results and Discussion

At the end of the trial, fish fed with 10% and 15% lipid diets at 28°C exhibited significantly higher final body weights compared to those fed the same diets at 22°C. A similar trend was observed for specific growth rate (SGR), with the exception that fish fed with 10% lipid diet at 22°C showed no significant differences when compared to those fed either lipids level at 28°C or the 15% lipid diet at 22°C. These findings indicate that fish reared at 28°C exhibited enhanced growth compared to those at 22°C, likely due to the warmer temperature being closer to the range (26–27°C) where improved growth performance has been reported in previous literature (Crosetti and Blaber, 2015)commonly known as grey mullets, are one of the most ubiquitous teleost families in coastal waters of the world. They occur in most temperate, sub-tropical and tropical waters in both hemispheres. As a family they have an extraordinary adaptability, which has resulted in species that are found mainly in the clear and pristine waters of coral reefs to those that prefer highly turbid estuarine and freshwaters . Some species can even survive in some of the most polluted waters in the world, e.g. in the harbour at Viskhapatnam in India (Blaber 2000. At 28°C, fish were presumably able to more efficiently utilize dietary nutrients to meet their energy demands, thereby supporting improved growth performance. In contrast, the reduced energy expenditure at 22°C may have limited nutrient utilization and growth. Regarding feed conversion ratio, fish fed with 15% lipid diet at 28 °C showed a tendency toward a lower FCR compared to all other treatments. However, fish fed with 10% lipid diet at 28 °C didn't show significant differences in FCR across the different lipid levels or temperature conditions. Moreover, feed intake was significantly lower in fish fed with 15% lipids at both temperatures compared to those receiving the 10% lipid diet. These results suggest that flathead grey mullet exhibited superior feed conversion efficiency with the 15% lipid diet at 28°C, indicating minimized feed waste and enhanced nutrient utilization.

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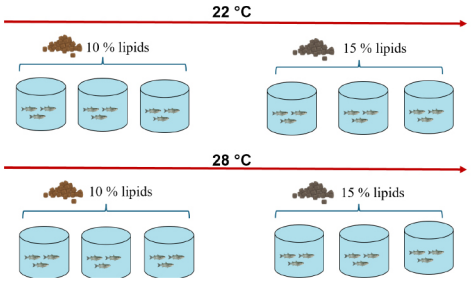


Figure 1. Experimental design of the trial: two diets differing in lipid content (10% and 15%) were tested at two temperatures (22°C and 28°C) in flathead grey mullet.

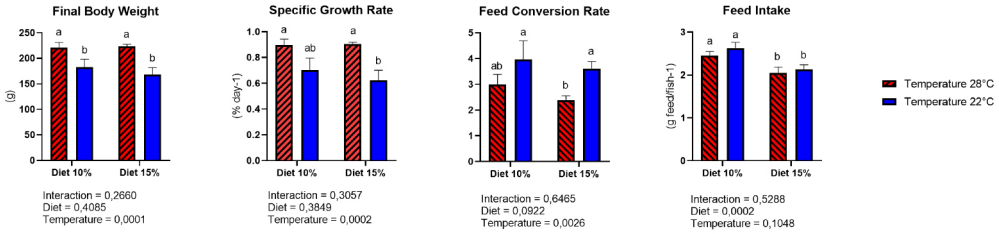


Figure 2. Final body weight, specific growth rate, feed conversion rate and feed intake of flathead grey mullet after a 100-day feeding trial conducted at two different temperatures (22°C and 28°C). Different letters indicate significant difference (two-way ANOVA $p \leq 0.05$ followed by Tukey's test) between treatments and temperature.

## Conclusion

In conclusion, the growth of the mullet at 28°C remained consistent across both dietary treatments. However, considering feed conversion ratio and feed intake, fish exhibited improved growth when provided with a diet containing 15% lipids at this temperature. A comprehensive understanding of the protein and energy requirements of this emerging species at varying temperatures is essential for optimizing growth performance and developing effective aquaculture practices.

## Acknowledgements

The project has received funding from the PRIN: progetti di ricerca di rilevante interesse nazionale – bando 2022; prot. 2022snlr4y. Mugil cephalus (linneus, 1758): the flathead grey mullet as a model for sex determination in fish with implications for aquaculture (acronym mugilsex)

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# COMBINING NUTRITIONAL AND ENVIRONMENTAL STRATEGIES TO ENHANCE FEEDING BEHAVIOR AND GROWTH IN FLATHEAD GREY MULLET *Mugil cephalus*

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**Introduction**
Flathead grey mullet (*Mugil cephalus*) is a catadromous, omnivorous fish species that has recently attracted growing scientific interest due to its potential as a sustainable, low-trophic-level option for aquaculture. In the context of a rapid global changing understanding how temperature and different environment interact is crucial for improving aquaculture sustainability. This abstract summarises preliminary findings from two growth trial conducted on flathead grey mullet, aimed at assessing the influence of temperature (Trial 1, T1) and sand-based environmental enrichment (Trial 2, T2).

**Materials and Methods**
(T1) Two isoproteic experimental diets were formulated to contain two lipid levels (Diet 10% and Diet 15%) and were tested at two temperatures: 22 °C and 28 °C. Diets were administered to triplicate groups of 30 fish each (initial weight: 90.5 g) over a period of 100 days at both temperatures. (T2) An experimental diet was formulated to contain low fishmeal (3%) and soybean meal (10%) levels. Diets were administered to triplicate groups of 18 fish each (initial weight: 124.5 g) over a period of 60 days at both different environmental enrichment (three of these tanks had been filled with 15 kg of sand each).

During both trial, feed was provided to apparent satiation during a single 6-hour meal per day and dissolved oxygen was maintained above 7.5 mg $L^{-1}$. At the end of the trial, final body weight (FBW), specific growth rate (SGR), feed intake (FI), and feed conversion rate (FCR) were calculated. Differences among treatments and temperatures were considered significant at $p < 0.05$ (one or two-way ANOVA followed by Tukey's test).

**Results and Discussion**
Environmental conditions significantly influenced fish growth performance in both trials. In T1, conducted at two water temperatures (28 and 22°C) and with two dietary lipid levels (10% and 15%), higher temperature resulted in increased final body weight, specific growth rate (SGR), along with improved feed conversion rate (lower FCR values). In T2, conducted with two environmental enrichments, the presence of sand appears to significantly modulate final body weight and specific growth rate showing also a tendency for a lower feed conversion rate. In both T1 and T2, feed intake was not influenced by environmental conditions. Results from this study indicate that flathead grey mullet exhibits strong tolerance to elevated water temperatures, including levels comparable to those recorded during heatwaves in Mediterranean regions. Moreover, environmental enrichment with sand, based on the observation that wild individuals often have sand present in their stomach contents, was associated with improved feed conversion rates (Whitfield et al., 2012). This suggests a possible correlation between natural substrate availability and digestive efficiency.

**Conclusion**
Although the flathead grey mullet is not considered a high-value species, its wide environmental adaptability and potential for sustainable production make it a promising candidate for diversified aquaculture systems. The development of rearing protocols that minimise environmental impact while supporting biodiversity will be essential for ensuring that future aquaculture practices remain both productive and ecologically responsible.

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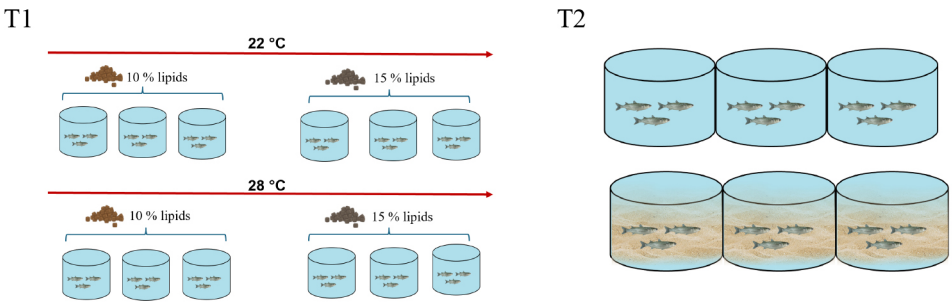


Figure 1. Experimental design of T1: two diets with different lipids inclusions (10% and 15%) were tested at two temperatures (22°C and 28°C) in flathead grey mullet. Experimental design of T2: different environmental enrichment (with or without sand) were tested on flathead grey mullet fed the same experimental diet.

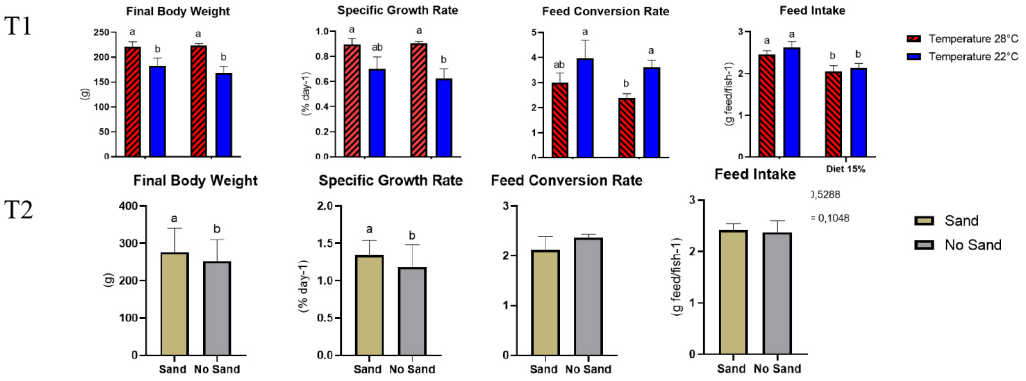


Figure 2. Final body weight, specific growth rate, feed conversion rate and feed intake of flathead grey mullet at two different temperatures (22°C and 28°C) (T1) and at two environmental enrichments (with or without sand) (T2). Different letters indicate significant difference.

**Acknowledgements**

The project has received funding from the PRIN: progetti di ricerca di rilevante interesse nazionale – bando 2022; prot. 2022snlr4y. Mugil cephalus (linneus, 1758): the flathead grey mullet as a model for sex determination in fish with implications for aquaculture (acronym mugilsex)

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# REWILDING PIKEPERCH: NATURAL DIETS AS PHYSIOLOGICAL TOOLS FOR PIKEPERCH (*Sander lucioperca*) RESTOCKING

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## Introduction

The successful restocking of pikeperch (*Sander lucioperca*) into natural habitats is a crucial strategy for both fisheries management and the conservation of this ecologically and economically valuable species (Lyutikov et al., 2024). Pikeperch, as an apex freshwater predator, exhibit pronounced phenotypic plasticity in response to environmental and dietary changes (Pourhosein-Sarameh and Falahatkar, 2024). However, transitioning from controlled aquaculture environments, often characterized by artificial diets and stable conditions, to the complex and variable wild poses significant physiological and behavioral challenges for RAS reared fish (Kozłowski and Piotrowska, 2024). One promising approach to enhance post-release survival is to reintroduce natural prey into the diet before restocking. These dietary interventions may facilitate the re-acclimatization of sensory systems, enabling more effective prey detection and capture in the wild.

## Material and methods

Five experimental groups were established, each maintained in a separate tank (one tank per group; five tanks in total) for a period of 30 days. All dietary treatments, including granular feed, granular feed with vitamins, and prey fish feed (administered for 10, 20, and 30 days), were provided ad libitum. All tanks were monitored under identical environmental conditions. At the end of the 30-day period, blood samples were collected, dissected to obtain liver, spleen, and gonad tissues, and all samples were processed for subsequent analyses. Blood biochemical parameters were measured using the Catalyst one analyzer.

## Results

An increasing trend in weight was observed across the five dietary groups (Gr, GrV, P10, P20, and P30), suggesting that all feeds adequately supported metabolic requirements ($p > 0.05$). In terms of blood biochemistry, cholesterol levels were significantly higher in the prey fish-fed groups (P10, P20, P30) compared to the granular control (Gr) and the vitamin-supplemented granular feed (GrV) ($p < 0.05$). Triglyceride levels showed an overall decreasing trend from the granular to prey fish-based diets, with statistically significant differences observed between Gr and P20 ($p < 0.05$), and between GrV and both P20 and P30 ($p < 0.05$). Although amylase activity did not follow a consistent trend, GrV showed significantly different values compared to the prey fish-fed groups (P10, P20, P30) ($p < 0.05$). Lactate levels decreased progressively across the groups, with Gr showing a statistically significant difference from P20 and P30 ($p < 0.05$). Glucose levels exhibited a declining trend across the treatments ($p > 0.05$). Ammonia levels showed no clear trend, but Gr differed significantly from P20 ($p < 0.05$). Additionally, significant associations were observed between glucose and ammonia, as well as between lactate and ammonia, particularly when comparing prey fish-fed versus non-prey fish-fed groups ($p < 0.05$)(Figure 1). No statistically significant differences were observed in the hepatosomatic index (HSI), viscerosomatic index (VSI), or gonadosomatic index (GSI) across the dietary groups ($p > 0.05$).

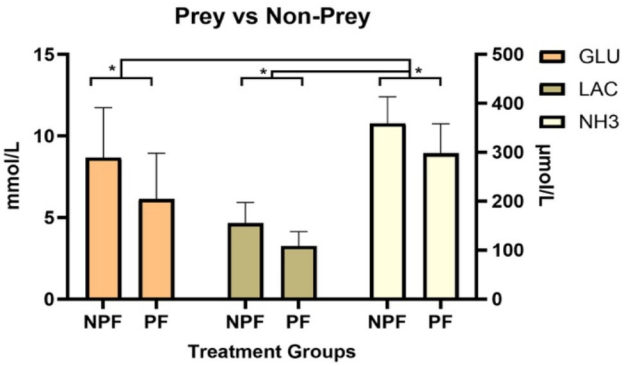


*Figure 1-Plasma glucose (GLU), lactate (LAC), and ammonia ($NH_3$) levels in non-prey (NPF) and prey fish (PF). * symbol stands for significant differences between different groups*

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**Conclusion**

The study found that all dietary treatments supported comparable pikeperch growth, but prey fish feed and granular diets differed significantly. Prey-fed groups had higher cholesterol and altered triglyceride levels, suggesting enhanced lipid metabolism consistent with natural prey consumption. Reduced lactate concentrations indicated improved anaerobic metabolic efficiency, which could be advantageous for wild reintroduction(Kestemont and Henrotte, 2015). Higher HSI tendency in certain groups reflected better liver health and potential resilience (Zhao et al., 2024). Integrating natural prey improves the likelihood of successful adaptation and survival in the wild.

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# GENERATION AND CHARACTERISATION OF A MUTANT STRAIN OF THE GREEN MICROALGAE *Chlamydomonas reinhardtii* WITH INCREASED TOLERANCE TO HEAT AND LIGHT STRESS

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Microalgae hold significant potential in biotechnology as a source of animal feed, high-value compounds, biofertilizers, and biofuels. Their high lipid and protein content makes them particularly valuable in aquaculture, where they are used either as live feed for fish and crustacean larvae or juveniles, or as ingredients in formulated feed.

However, the productivity of microalgae is often constrained by environmental factors such as light and temperature stress. Global warming and climate change further exacerbate these challenges, significantly compromising the biotechnological potential of microalgae. In this study, we aim to develop genetically modified microalgae strains with enhanced tolerance to elevated temperatures and light intensities, thereby improving their applicability in aquaculture and other green technologies. As a model organism, we employ the green alga *Chlamydomonas reinhardtii*, widely used in studies of microalgal genetics, physiology and ecology.

By combining random insertional mutagenesis with high-temperature selection, we have generated several mutant strains of *C. reinhardtii* exhibiting enhanced tolerance to heat stress. One strain, in particular, displays the highest level of thermotolerance – up to 38°C– compared to the WT strain, this phenotype being maintained under high light intensity. Functional and metabolomic studies revealed changes in photosynthetic efficiency as well as in redox and bioenergetic metabolism in the mutant strain. The mutated gene has been identified as encoding a membrane transporter. Comparative transcriptomic analyses between the mutant and WT strains are being conducted. Additionally, physiological assays and subcellular localization studies are ongoing, to elucidate the mechanisms underlying the enhanced thermotolerance observed in this mutant strain.

This work was supported by the "Junta de Andalucía" (grant PCM_00004, "Plan de Recuperación, Transformación y Resiliencia" –funded by the European Union–NextGenerationEU).

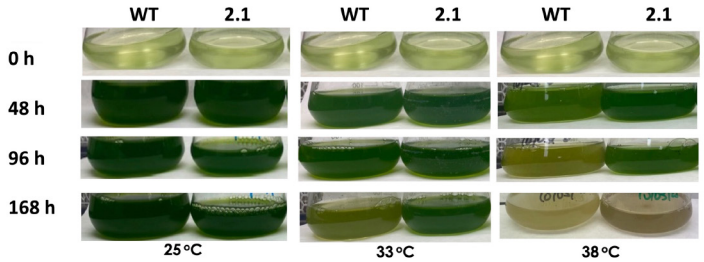


**Figure 1.** Examples of individual cultures of wild-type (WT) and 2.1 mutant strains grown at different times at an illumination intensity of 60 µE $m^{-1}$ $s^{-2}$ under different temperature conditions, as indicated.

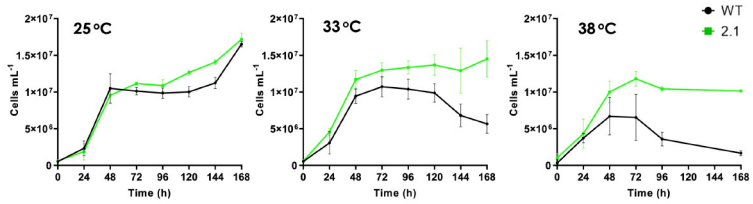


**Figure 2.** Growth curves of wild-type (WT, black) and 2.1 mutant (green) strains cultures grown at 60 µE $m^{-1}$ $s^{-2}$ under different temperatures conditions, as indicated.

# THE CROSSTALK BETWEEN DIETARY RAYON MICROFIBERS AND THE FISH HOLOBIONT: IMPACTS ON GUT, SKIN, AND ENVIRONMENTAL MICROBIOTA

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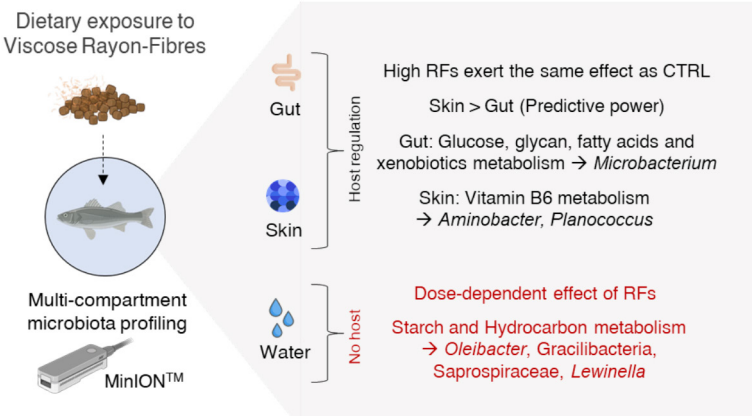
## Introduction

Viscose-rayon microfibres (RFs) are polymers derived from the textile industry that are widely dispersed throughout aquatic environments worldwide. Whether ingested or simply present in the surrounding environment, their presence would affect both wild and farmed animals (Matias et al., 2024a). Thus, a recent study on European sea bass (*Dicentrarchus labrax*) showed that the increased presence of RFs in aquafeeds (CTRL – no RFs; RF1 – 0.001 g/kg; RF2 – 0.01 g/kg; RF3 – 0.1 g/kg) was linked to an exponential increase in RF particles in the water, ranging from none in the tanks with CTRL-fed fish to 0.40 ± 0.21 RFs/L in those with fish fed the highest inclusion level (RF3) (Matias et al., 2024b). This increased RF release and exposure was associated to a fatty liver without signs of growth impairment in the short term. However, a multi-tissue, multivariate gene expression analysis identified up to 19 discriminant genes, which depicted changes in hepatic lipogenic enzymes and intestinal/head kidney inflammatory markers, among others. Besides, these expression patterns denoted opposing regulation of immune responses at the systemic and local levels, which contributed to distinguishing CTRL_RF1 from RF2_RF3 fish. To further explore other disruptive physiological processes, this study aimed to assess the impact of RF exposure on the host and associated microbial communities, employing a multi-layered approach for the integrative profiling of gut, skin, and environmental water microbiota.

## Materials and methods

European sea bass juveniles (5.8 g) were distributed in triplicate groups (180 fish per dietary treatment) in an flow through system. Fish were fed for 68 days to visual satiety with CTRL, RF1, RF2 and RF3 diets near to the visual satiety with automatic feeders (3 times per day). At the end of trial, 9 overnight fasted fish per diet were randomly sampled for gut and skin microbiota. Water samples (1L) were taken from the outlet water flow (OW) of each tank with fish, and from the inlet water flow (IW) from tanks without fish. Sequencing libraries were loaded in a MinION device, amplifying the full-length 16S rRNA gene with universal primers, using recently standardized sequencing procedures (Domingo-Bretón et al., 2024). Bioinformatic analyses included demultiplexing, quality filtering and taxonomy assignment. Richness and alpha-diversity were calculated in R. Partial least squares-discriminant analysis (PLS-DA) was used to depict the separation of experimental groups to be validated through permutation analyses (500 permutations). A minimum VIP value for the correct clustering of the 80% of samples was fixed in any microbiota layer, being 1.6, 1.3 and 1 the thresholds established for AI, skin and water, respectively. Bacteria taxa overcoming this threshold and displaying a high relative abundance (>0.5%) were selected as the main drivers. Inferred metagenome analysis was performed with Picrust2 tool and significant genes were considered at an FDR < 0.05.

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**Results and discussion**

A total of 95,000 reads per sample were assigned to 978 taxa in at least one of the three microbial compartments. In both the gut and skin, dietary RF exposure decreased bacterial richness at low and intermediate doses (RF1, RF2), while similar values were found in CTRL and RF3 fish. In water samples, RF diets did not significantly alter microbiota richness, although the general trend was a decrease in bacterial diversity in RF2 and RF3 that reached similar values to those found in IW samples. Overall, these findings highlight that the presence of RFs in fish feeds can alter the microbiota of both the host and its surrounding aquatic environment, with different allosteric loads that seem to require a certain threshold level for activation. This assumption is corroborated by PLS-DA analysis, which clustered the AI and skin samples of the CTRL and RF3 groups together, while in water samples, the clustered groups were CTRL_RF1 and RF2_RF3. Discriminant analysis further revealed that the number of bacterial taxa with a discriminant value increased exponentially from AI (27 taxa) to skin (141) and water (792), again reflecting a different allosteric capacity – higher in animal mucosal environments than in the aquatic environment. Additionally, this buffering capacity appeared greater in the gut environment compared to the skin, the latter being more susceptible to environmental fluctuations due to its open nature. Upon applying an abundance filter, the number of discriminant bacteria was reduced to 3, 6, and 13 in AI, skin, and water, respectively. Inferred metagenomic analysis indicated that the abundant gut bacteria (*Microbacterium* and Alcaligenaceae spp.) are primarily involved in the activation of polysaccharide and xenobiotic metabolism, whereas predominant skin bacteria (*Aminobacter* and *Planococcus*) are associated with vitamin B6 biosynthesis. In the water microbiota, up to 28 metabolic pathways appeared altered as a result of changes in taxa abundance, with particular relevance to starch and hydrocarbon metabolism through the action of *Oleibacter*, Gracilibacteria, Saprospiraceae, and *Lewinella* taxa.

**Concluding remarks**

This integrated perspective highlights the multifactorial nature of environmental pollution, microbial ecology, and host-microbe interactions, suggesting novel bacteria taxa and pathways through which microplastics exposure can influence host metabolic regulation.

**Funding**

This work was supported by the TNA programme (PID: 22708 and 25209) within H2020 AQUAEXCEL3.0 project (871108) to R. Matias for accessing to IATS-CSIC facilities.

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# HARNESSING THE POTENTIAL OF *Nannochloropsis* GROWN ON BREWERY WASTEWATER AS A SUSTAINABLE FEED FOR SEA BREAM

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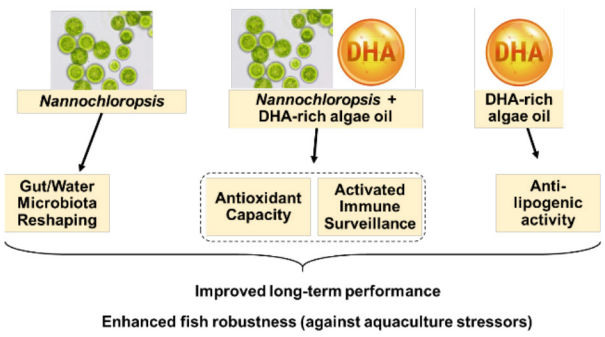
## Introduction

Microalgae are increasingly recognized as a sustainable solution to environmental challenges, particularly in the field of wastewater treatment. Certainly, these remarkable microorganisms are able to extract excess nutrients from wastewater and transform them into high-value biomass - eco-friendly resource - that can be turned into nutritious aquaculture feed. Among various microalgae, *Nannochloropsis* stands out due to its robust growth, high protein and lipid content, and significant levels of polyunsaturated fatty (Han et al., 2019). However, the in-depth potential of *Nannochloropsis* for the valorisation of waste products (e.g., brewery waste) into aquafeeds remains largely unexplored. The aim of this study was to investigate some of the physiological consequences in fish of the use of such microalgae-based production systems with focus at the systemic, tissular and environmental level from a holobiont perspective (i.e., the interconnected system of the host and its associated microorganisms), using gilthead sea bream as a model of Mediterranean farmed fish.

## Materials and methods

The potential of *Nannochloropsis* diets to revalorize brewery waste was analysed in a 96-days gilthead sea bream trial from April to July under natural temperature and daily photoperiod at the IATS latitude (40° 5' N; 0° 10' E). The experimental design with triplicate tanks per dietary treatment (210 fish per diet, 11 g initial BW) included four diets: a commercial-based diet as a negative control (D1), and three fishmeal-free diets with varying inclusion levels of *Nannochloropsis oceanica* (D2, 0%; D3, 2%; D4, 14%). For the most extreme diet formulation (D4), fish oil was replaced by a DHA-enriched microalgae oil, resulting in a diet devoid of fishmeal and fish oil and with an EPA+DHA content (1.80%) above the established nutrient requirements for juveniles of gilthead sea bream. All four diets were formulated by LSAqua (Hooglede; Belgium) to be isoproteic (48% dry matter) and isolipidic (18% dry matter) feeds with different inclusion levels of fishmeal, fish oil, plant proteins and other alternative ingredients (soya oil, poultry meal, processed animal proteins, single cell proteins, and microalgae biomass) to facilitate aquaculture eco-intensification through increased circularity and resource utilization. At the end of trial, 12 fish per dietary treatment were sampled for analyses of total plasma antioxidant activity, host gene expression, and bacteria composition of the adherent mucus layer and water environment. Antioxidant capacity was measured as Trolox activity. Gene expression was made using customized tissue-specific arrays with 89 selected markers of growth, lipid metabolism, antioxidant defence, and immunological status. Microbial characterization was performed amplifying full-length 16S rRNA using Oxford Nanopore sequencing. Bioinformatic analysis comprised demultiplexing, quality filtering and taxonomic assignment. Statistically significant differences ($p < 0.05$) on growth performance, and gene expression were assessed by one-way ANOVA/Kruskal-Wallis test followed by a Holm-Sidak *post-hoc* test.

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**Results and discussion**

All fish groups grew efficiently and only slight differences on final weight (< 5%) were found between D1-fed fish and the other three experimental groups fed fishmeal-free diets (D2-D4), which remained almost undistinguishable in terms of growth and feed conversion. However, fish fed the D4 diet showed an up-regulated hepatic expression of two key endocrine regulatory factors, *igf1* and *ghr1*, suggesting an improved growth potential at the transcriptional level in fish fed with the highest microalgae inclusion level. No signs of histopathological damage were found in the liver and intestines of any experimental group, but D4 fish displayed a reduced lipid vacuolization of enterocytes accompanied by a depletion of hepatic lipid droplets. Certainly, PLS-DA highlighted a consistent down-regulation of genes involved in fatty acid synthesis, such as desaturases (*scd1a*, *fads2*), elongases (*elovl1*, *elovl5*, *elovl6*), and fatty acid transporters (*hfabp*), alongside the up-regulation of hepatic lipase (*hl*). This shift is indicative of a metabolic adaptation that favours tissue-fatty acid uptake over de novo fatty acid synthesis. Likely this metabolic feature was primarily due to the highest PUFA content (DHA-rich algae oil) of D4, while the increased plasma antioxidant capacity of D3 and D4 fish would be the result of a combination of enzymatic and non-enzymatic antioxidant mechanisms that become activated with the increased dietary supply of PUFA and microalgal biomass. The microalgae-based diets also modulated the expression of several intestinal genes, including interleukins, galectin binding receptors, brush border enzymes, and markers of tight junction integrity (*il1b*, *il34*, *tnf□*, *lgals*, *alpi*, *tjp1, csflr1*, *cxadr*), encompassing both pro- and anti-inflammatory roles. Such finding denotes a delicate balance in the host intestinal transcriptome of D3-D4 fish, which helps to avoid excessive inflammatory responses while keeping the immune system alert to prevent the entry of invading pathogens. Such adaptive gene expression pattern would be favoured by the reshape of gut microbiota with an overrepresentation of 17 bacterial genera with potential detoxifying, antioxidant and anti-inflammatory properties. Among them, up to seven bacteria genera were markedly overrepresented in the intestine of D4 fish (*Vibrio, Hydrogenispora, Marinobacter, Palleronia-Pseudomaribius, Ruegeria, Atopostipes, Thaumasiovibrio*). The microbiota of the water environment was also altered by the dietary treatment, denoting the presence of active feeding fish, changes in diet composition and, more specifically, the degree of *Nannochloropsis* supplementation.

**Concluding Remarks**

*Nannochloropsis* supplementation largely reshaped the microbiota inhabiting the intestine and the water environment, promoting in turns a healthy intestinal core microbiota. Microalgae biomass, together with DHA-rich algae oil, would drive the expression pattern of key enzymes of lipid metabolism, antioxidant defence and immunological surveillance, while changes in lipid metabolism could be primarily attributed to a dietary surplus of PUFA rather than to an increased supply of microalgal biomass.

**Funding**

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# SOCIO-ECONOMIC AND ECOLOGICAL IMPACTS OF FORAGE FISH USAGE IN FISHMEAL: A CASE AGAINST INEQUITIES IN FISHERIES AND AQUACULTURE£

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**Introduction**

Forage fish are essential for food security in developing countries and fulfill critical roles in marine ecosystems. However, 20 to 30 million metric tons of forage fish are harvested yearly in low-income countries for use in the fishmeal and aquafeed industries (Fig.1, depicting catch allocation trends), mainly catering to developed countries. This exploitation of fish diminishes the supply to fresh fish markets, escalating local fish prices and marginalizing artisanal fishermen and their communities in leading fish-producing nations.

Ecological consequences are equally troubling—overfishing disrupts marine food webs, depleting piscivores like mackerel, seabirds, and marine mammals. The ethical implications of such practices are profound, particularly for low-income populations in forage-fish-producing nations (Fig. 2, illustrating the main stakeholders in the forage fish industry). Rising fish prices diminish access to nutritious diets and create financial strain for millions in fish-producing nations (Fig. 3, typical fish markets in low-income communities; removing fish from this market to the industry raises prices for the local consumers).

**Analysis**

Our research evaluates these trends' financial and nutritional effects, exploring the financial impact of a hypothetical 10% drop in fresh forage fish allocation to local markets for illustrative purposes. The findings suggest that even such a slight fish supply shift could significantly impact consumers' expenses by hundreds of millions of dollars globally and tens of millions of dollars nationally, as well as increase the catch of larger fish and significantly improve household nutrition in underprivileged communities.

We advocate for industry and policy reforms to rectify these inequities, including promoting alternative aquafeed protein sources and cultivating non-carnivorous fish. Only by prioritizing equitable access can the fisheries industries ensure that forage fish resources serve both human and ecological well-being.

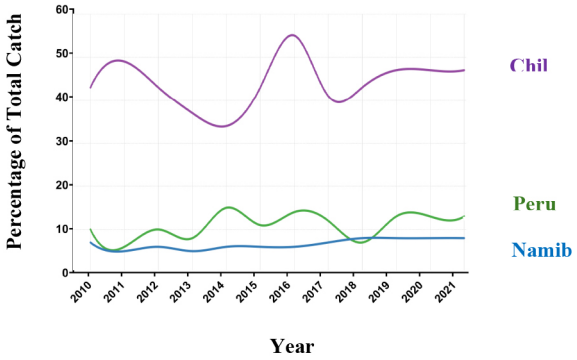


Fig. 1: Trends in forage fish catch fraction sold in the fresh fish markets, most of the catch goes to the fishmeal and aquafeed industries (2010–2021).




Figure 2: Key stakeholders and issues related to forage fish fisheries in this presentation. Overlooking the needs of poor consumers, fishermen, and the ecosystem gives undue advantage to the fishmeal and aquafeed industries.

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Fig. 3a: A bustling fish market in Kavieng, New Ireland, Papua New Guinea, showcasing the variety of both forage and larger fish available in the local economy; 3b: A fish market featuring several kinds of forage fish in Madagascar (Photos: M. Agami)..

**Reference**

[£] More details: Neori, A. and Agami, M., 2024. Low-Income Fish Consumers' Subsidies to the Fish Reduction Industry: The Case of Forage Fish. World, 5(3), pp.769-788. https://doi.org/10.3390/world5030040

# EFFECTS OF SIZE GRADING ON GROWTH PERFORMANCE, SURVIVAL AND SIZE DISTRIBUTION OF WHITELEG SHRIMP (*Litopenaeus vannamei*) IN AQUACULTURE

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## Introduction

Size grading is an established practice for a variety of aquaculture species. A more homogeneous size distribution often reduces cannibalism and allows optimal feed management. It was indicated that size grading also positively influences the growth performance of *L. vannamei* and could be implemented in a farming environment (LI et al., 2010). Nowadays, the most established grading techniques for shrimp in aquaculture are hand sorting and screening, which are rather invasive and labor-intensive. Possible positive effects on the survival and growth of the smaller shrimp after sorting are mostly unknown.

The aim of the present experiment was to evaluate the effects of size grading on growth performance and survival of small shrimp, large shrimp and unsorted shrimp groups.

## Material and Methods

After a 14-days acclimation period, a batch of shrimp (0.4 ± 0.6 g) sourced from Aquapurna, Germany, was graded into two different size categories (small and large) using a sorting grid with a 4.7 mm opening size. Additionally, a not sorted shrimp group was used as the unsorted control (CU). The sorted animals were divided into three treatments: 1. Small Sorted (SS, 0.1 ± 0.0 g); 2. Large Sorted (LS, 1.0 ± 0.1 g) and 3. Control Sorted (CS, 0.5 ± 0.1 g). For the CS, the initial ratio of small to large animals was calculated after sorting and the animals were recomposed following that exact ratio. Afterwards, shrimp from each treatment were randomly selected, photographed, weighed, and stocked into a 100 L tank. Each treatment had four replicates, adding up to 16 tanks in total with 20 shrimp per tank. The shrimp were fed following the Le Gouessant "VANNA RANGE" feeding table. After 57 days, the experiment was finalized and all shrimp were weighed and measured.

## Results and Conclusion

The total biomass, average body weight (ABW) and coefficient of variation (CV) of the initial sampling (day zero) are shown in Table 1.

After 57 days, all treatments showed a significantly higher final biomass than the SS treatment (Holm-Sidak, $p < 0.001$). While the LS treatment had the highest average biomass, the differences were not significant compared to CU and CS. Similar results were shown for the biomass gain and the average body weight. The SS treatment had the highest SGR (6.3 ± 0.2 %) which was significantly higher than all other treatments. There were no significant differences between the survival rates of the different treatments (One-way ANOVA, $p = 0.481$) (Table. 2).

The results indicate that while small shrimp are able to partially compensate for the initial size difference due to their higher SGR, full compensatory growth could not be achieved through size grading. Nevertheless, size grading can positively impact the production of *L. vannamei* by increasing the uniformity. The CV of the large sorted shrimp group was lower compared to the controls. While slightly increasing the total biomass production, a lower CV additionally results in harvesting a more homogeneous and therefore higher-value product. It lowers the risk of harvesting undersized shrimp that cannot be sold or have to be sold at a much lower price. Animals that are too small can be removed from the system in an early stage, saving resources and increasing profits.

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*Table 1: Initial biomass, average body weight [ABW] and the coefficient of variation [CV] for Unsorted Mixed Shrimp Group (CU), Sorted Mixed Group (CS), Small Sorted Group (SS) and Large Sorted Group (LS). N=4*

| Treatment | Biomass [g] | ABW [g] | CV [%] |
|---|---|---|---|
| **CU** | 9.3 ± 1.6 | 0.5 ± 0.1 | 128 ± 10.0 |
| **CS** | 10.6 ± 1.3 | 0.5 ± 0.1 | 97.6 ± 11.4 |
| **SS** | 2.8 ± 0.3 | 0.1 ± 0.0 | 91.2 ± 17.8 |
| **LS** | 20.0 ± 2.6 | 1 ± 0.1 | 64.8 ± 7.3 |

*Table 2: Effects of size grading on different size groups of L. vannamei after 57 days of cultivation. Treatments: CU = control unsorted, CS = control mixed group after sorting, SS = small sorted shrimp, LS = large sorted shrimp; BG = biomass gain; ABW = average body weight, CV = coefficient of variation; SGR = specific growth rate, SR = survival rate. N = 4, α = 0.05*

| Trt. | Biomass [g] | BG [g] | ABW [g] | CV [%] | SGR [%] | SR [%] |
|---|---|---|---|---|---|---|
| **CU** | 95.2 ± 6.3$^{a}$ | 85.9 ± 6.4$^{ab}$ | 10.0 ± 1.3$^{a}$ | 50.9 ± 12.6$^{ab}$ | 5.4 ± 0.2$^{a}$ | 48.0 ± 8.3$^{a}$ |
| **CS** | 96.7 ± 20.3$^{a}$ | 86.1 ± 19.1$^{ab}$ | 8.6 ± 0.6$^{a}$ | 55.7 ± 10.5$^{b}$ | 4.9 ± 0.2$^{b}$ | 56.3 ± 12.4$^{a}$ |
| **SS** | 60.8 ± 6.8$^{b}$ | 57.9 ± 6.6$^{b}$ | 5.1 ± 0.4$^{b}$ | 60.5 ± 8.7b | 6.3 ± 0.2$^{c}$ | 59.3 ± 6.3$^{a}$ |
| **LS** | 115.2 ± 15.7$^{a}$ | 95.2 ± 14.4$^{a}$ | 10.0 ± 0.3$^{a}$ | 28.7 ± 3.1$^{a}$ | 4.1 ± 0.2$^{d}$ | 56.8 ± 8.2$^{a}$ |

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# EFFECTS OF L-TYROSINE, L-PHENYLALANINE AND L-TRYPTOPHAN ON THE INDUCTION OF METAMORPHOSIS IN *Ruditapes decussatus* LARVAE

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## Introduction

The grooved carpet shell, *Ruditapes decussatus*, is a high-value bivalve, native to European and North African coasts and widely targeted by fisheries and aquaculture. Successful hatchery production remains limited by larval settlement and metamorphosis, stages that represent the transition from planktonic free-swimming to benthic juveniles. Across marine invertebrates, settlement and metamorphosis are triggered by biological, chemical and physical factors. Chemoreceptors detect these environmental cues and activate neural circuits that drive the morphological and behavioural changes associated with larval metamorphosis. In bivalves, neuroactive compounds, such as catecholamines (e.g., epinephrine) and neurotransmitters (e.g., serotonin), have been widely investigated for their capacity to induce larval metamorphosis[1]. These compounds have been used to induce, synchronise or accelerate the metamorphosis of different molluscan species. Catecholamines and some neurotransmitters have some amino acids as precursors. For instance, L-tyrosine and L-phenylalanine are precursors in catecholaminergic pathways, while L-tryptophan is a serotonin precursor. Therefore, this study explores the ability of these specific amino acids – L-tyrosine, L-phenylalanine, and L-tryptophan – to act as metamorphic inducers in *R. decussatus* larvae under controlled conditions.

## Material and Methods

This study evaluated the effects of different concentrations of L-tyrosine (TYR), L-phenylalanine (PHE) and L-tryptophan (TRYP) ($10^{-4}$, $10^{-5}$, and $10^{-6}$ M) and exposure times (24, 48, and 72 hours) on the metamorphosis of *R. decussatus* larvae. The larvae were reared in filtered and sterilised seawater and fed daily with a bispecific diet of *Tisochrysis lutea* and *Chaetoceros calcitrans*. Larvae were considered competent for settlement after being retained twice on a 150 µm sieve (approximately 18 days). The experiments were conducted in glass beakers with 300 mL of clean seawater (FSW) with stimuli. Each treatment beaker contained a sieve (150 µm mesh) with approximately 1000 larvae. Beakers were under continuous aeration and a constant temperature (21ºC) and salinity (33.2). After each exposure period, larvae were removed from the test solution, rinsed with FSW, observed under a microscope, and placed back into the beakers filled with FSW until completing 6 days of the experiment. Metamorphosis percentage, validated by the presence of branchial arches, and survival rate were assessed after each exposure period and at the end of the experiment.

Results were analysed with R 4.4.1 software. Percentages of metamorphosed larvae and mortality were analysed using the Aligned Rank Transform (ART) method, followed by Tukey's *post-hoc* test. This non-parametric approach enables testing the main effects and interactions by aligning and rank-transforming the data[2], followed by standard ANOVA. Statistical significance was set as $p < 0.05$.

## Results

The effects of the different chemical treatments on larval metamorphosis after 6 days of experiment are represented in Fig. 1. Exposure during 24 hours of TYR $10^{-5}$M and $10^{-4}$M, PHE $10^{-5}$M and TRYP $10^{-6}$M and during 48 hours of TYR $10^{-5}$M and PHE $10^{-6}$M induced a significantly higher metamorphosis rate than the respective control, with no significant differences. Exposure of 72h to all compounds, except TYR $10^{-5}$ M, PHE $10^{-5}$ M and $10^{-6}$ M and TRYP $10^{-6}$M, evoked a significantly lower percentage of metamorphosed larvae. TRYP at $10^{-4}$M was revealed to be lethal, with 100% mortality. Similarly, only less than 10% of larvae treated with PHE $10^{-4}$M survived. The 72-hour control showed less than 50% mortality, with the highest percentage of metamorphosed larvae. Other compounds did not influence the percentage of metamorphosis compared to the control.

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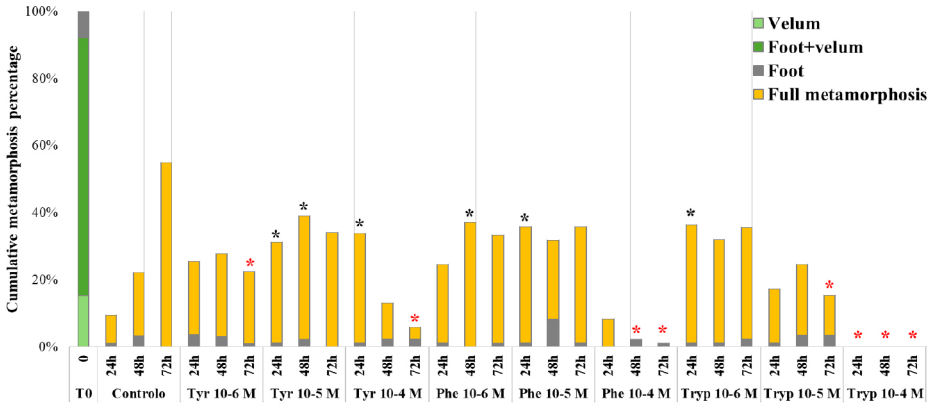


**Fig. 1**. Effects of L-tyrosine (Tyr), L-phenylalanine (Phe) and L-tryptophan (Tryp) exposure (24, 48 and 72 h) on larvae of *R. decussatus* at different concentrations ($10^{-4}$, $10^{-5}$ and $10^{-6}$ M) after 6 days. Column colours represent different development stages of larvae. Black (*) and red asterisks (*) indicate positive and negative significance differences against the control, respectively.

## Discussion and Conclusion

The present study demonstrates that L-tyrosine and L-phenylalanine had the best results in inducing metamorphosis in *R. decussatus* larvae. Both amino acids are biochemical precursors in catecholaminergic pathways, leading to the synthesis of dopamine, norepinephrine, and epinephrine, which have been implicated in metamorphosis induction in other bivalve species[1].

For L-tyrosine, the higher metamorphic and lower mortality rates occurred at $10^{-5}$M for 24–48 h or $10^{-4}$M for 24 h, indicating that a higher concentration may be required for receptor saturation over shorter exposures. In contrast, L-phenylalanine achieved similar effects at $10^{-6}$M for 48 h or $10^{-5}$M for 24 h, which may indicate that lower concentrations and exposure may be sufficient to induce a higher metamorphosis rate. L-tryptophan, a precursor in the serotonergic pathway, was found to be only efficient at low concentrations and short exposures and lethal at higher concentrations, with 100% mortality at $10^{-4}$M.

That precursors of catecholamines exhibited better results in inducing metamorphosis is in accordance with the literature, in which catecholamines are more suitable inducers for metamorphosis than serotonin[1]. The effectiveness of L-tyrosine and L-phenylalanine highlights their potential use as chemical inducers for metamorphosis. These amino acids are affordable and may be easily introduced in hatchery protocols optimisation.

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# NOVEL CAPACITY BUILDING APPROACH TO SUPPORT FUTURE COMPETENCE NEEDS IN THE AQUACULTURE SECTOR

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## Introduction

Knowledge is fundamental for developing smarter, greener, and more efficient solutions that foster societal trust and enhance the reputation of the aquaculture industry. Like many sectors, the seafood industry faces major challenges driven by climate change, environmental issues, resource constraints, and heightened demands for improved animal welfare, necessitating innovation and sustainable development. At the same time, Norwegian seafood companies anticipate employment growth and a substantial shift in skills needs toward 2030. Recent data from Møreforskning show that 60% of seafood companies report unmet competence needs, with half experiencing recruitment challenges. In addition to operational personnel, there is a growing demand for specialists including engineers, economists, biologists, lawyers, and data scientists.

## Materials and methods

To address these challenges, the regional public-private partnership Bridgehead Aquaculture, has been established. The initiative aims to align workforce competencies with sector demands through cross-level and interdisciplinary education. A key element involves connecting university students with pupils at vocational schools in coastal communities, promoting mutual understanding, sharing experience, and inspiring future careers within the blue sector.

Innovative educational models have been implemented, such as a two-semester practical aquaculture course for bachelor's and master's engineering students. It allows them to manage a complete fish production cycle, from fertilized egg to smolt, gaining deep insight into production processes, health management, welfare considerations, and environmental interactions. Students from diverse engineering disciplines learn to contextualize their expertise within aquaculture systems, enhancing both technical understanding and ecological awareness.

## Results

This cross-disciplinary approach is extended to PhD education, where candidates from technology, natural sciences, and humanities collaborate under NTNU's strategic research area Ocean and Coast - Strategic research area. By integrating interdisciplinary collaboration and real-world exposure into aquaculture education at all levels, the initiative enhances educational relevance, fosters targeted recruitment, and drives science-based innovation. This model contributes to preparing the next generation of specialists to address evolving sector challenges, supporting the sustainable growth of aquaculture aligned with societal values and environmental imperatives.

# HEAD KIDNEY LEUKOCYTES REMEMBER: MOLECULAR INSIGHTS INTO TRAINED IMMUNITY IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

The immune system in teleost fish, similar to other vertebrates, consists of both innate and adaptive immunity. Immunological memory has long been believed to be a unique characteristic of the adaptive immune system that generates specific and long-lasting responses to antigens. However, a novel understanding has emerged recently demonstrating that the innate immune system also possesses memory-like capabilities, a phenomenon referred to as "trained immunity". This is a significant finding because the innate immune system was previously considered a rapid but non-specific defence lacking immunological memory. Studies in fish have suggested that innate immune cells, such as macrophages and neutrophils, can exhibit an enhanced response upon re-exposure to stimuli, highlighting a new layer of complexity in host defence. Studying "trained immunity" in fish is particularly novel and relevant due to their evolutionary position as early vertebrates with a well-developed innate immune system. This capacity for innate immune cells to "remember" prior encounters, potentially through metabolic modulation and epigenetic reprogramming, offers a promising direction for understanding broad-spectrum protection and developing sustainable disease management strategies in aquaculture.

**Materials and Methods**

In this research, leukocytes were isolated from the head kidney of 100 g Atlantic salmon (*Salmo salar*) in freshwater stage and cultured under standardized conditions for 2 days. Cells were first stimulated for 24 hours with β-glucan (10 μg/mL) to induce a training response, followed by a five-day resting period in the same cultured condition. Subsequently, cells received secondary stimulation with phosphate-buffered saline (PBS, control), β-glucan (100 $\mu$g/mL, homologous stimulus), and lipopolysaccharide (LPS, 100 $\mu$g/mL, heterologous stimulus). Cell's immune activity was assessed by phagocytosis, nitric oxide (NO), and reactive oxygen species (ROS) production. To evaluate metabolic regulation, intracellular levels of lactate and fumarate were quantified. In addition, quantitative PCR was performed to assess gene expression related to immunity, glycolysis metabolism, and epigenetic regulation.

**Results**

Preliminary results showed that trained cells exhibited a noticeable enhancement in phagocytic activity upon secondary stimulation with both β-glucan and LPS compared to untrained cells. ROS production increased in β-glucan-stimulated cells, while NO levels remained relatively consistent among groups. Besides, fumarate concentrations were higher in β-glucan treatments and appeared to decline in trained groups, meanwhile, intracellular lactate levels remained relatively stable across both trained and untrained groups. Gene expression analysis of cells after the resting period showed elevated immune and metabolic gene expression in the trained group, with significant differences mainly in metabolic genes. While gene expression profiles were largely similar between trained and untrained leukocytes, several genes demonstrated distinct expression levels in the trained group, indicating potential targets of trained immune regulation. In addition, LPS stimulation elicited higher immune and metabolic gene expressions compared to PBS and β-glucan within the same group. In contrast, most epigenetic-related genes tend to exhibit minimal variation in expression levels across treatments and groups.

**Conclusion**

These findings provide preliminary evidence that β-glucan can induce trained immunity in Atlantic salmon leukocytes. The observed functional and metabolic shifts suggest that trained innate responses in fish may contribute to broad-spectrum immune protection. This study highlights the potential of trained immunity as a novel approach for improving disease resilience and supporting sustainable health strategies in aquaculture.

**Acknowledgements**

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# DISTANCE DECAY EFFECTS AND PUBLIC ATTITUDES TOWARDS AQUACULTURE AND MARINE ACTIVITIES IN IRISH COASTAL COMMUNITIES

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## INTRODUCTION

As global seafood demand continues to increase, aquaculture is playing an increasingly vital role in food security. Despite its potential, the sector's expansion often encounters resistance, particularly in coastal regions where marine space is contested (Hynes et al., 2018). This study examines public attitudes toward aquaculture in Connemara, Ireland, with a focus on the relationship between proximity to aquaculture operations and public perception of their environmental and socioeconomic impacts. Specifically, it explores the "distance decay" effect, offering insights to support evidence-based Marine Spatial Planning (MSP). Understanding the drivers of social acceptance is essential as Ireland aims to grow its blue economy in line with EU directives, while maintaining environmental integrity and community support.

## MATERIALS AND METHODS

Face-to-face surveys were conducted across Connemara, County Galway, between June and July 2019. A total of 245 fully completed questionnaires were collected, yielding a response rate of 67%. Participants aged 18 and older were interviewed in their homes, with a focus on the Galway Gaeltacht and adjacent areas.

The survey captured demographic data alongside respondents' awareness of aquaculture, environmental concerns, and preferences for future marine developments. Perceptions of threats to the marine environment and support for various marine activities were assessed using Likert-scale questions. Preferences for activity intensity were measured on a scale from 0 (no activity) to 5 (very high intensity).

Data were compared with findings from previous national surveys and a case study in Mulroy Bay, County Donegal. A logistic regression model was used to identify predictors of perceiving aquaculture as an environmental threat, including age, gender, education, proximity to aquaculture sites, and sub-regional location within Connemara.

## RESULTS AND DISCUSSION

The findings reveal strong local support for aquaculture in Connemara, with 73.7% of respondents supporting medium to high levels of activity in their local bay. Only 11% identified aquaculture as a significant environmental threat—considerably lower than in Mulroy Bay (17%) and the national average (40%). These results suggest that familiarity with aquaculture may mitigate negative perceptions.

Age emerged as a key demographic predictor: respondents under 35 were significantly more likely to express environmental concerns ($p < 0.05$), whereas those over 55 showed the highest levels of support for aquaculture expansion. Education level also influenced attitudes—university-educated individuals were more environmentally cautious than those with only secondary education (odds ratio = 1.72, $p < 0.05$).

Economic priorities were prominent among respondents, with 54% identifying employment as their top concern, compared to 41% prioritising environmental protection. This diverges from national patterns, where environmental concerns typically dominate. Notably, 84% agreed that "fish farming creates opportunities for local employment."

Significant regional differences emerged within Connemara. Support for aquaculture was highest in Carna (89%), followed by Cashel (76%) and Carraroe (64%). Regression results indicated that Carna was the only region where aquaculture was not perceived as an environmental threat (coefficient = -2.78, $p < 0.05$), likely reflecting the area's long-standing engagement with the sector. In contrast, respondents in Spidéal, Carraroe, and Clifden expressed stronger support for marine protected areas (coefficients = 35.55, 20.02, and 45.85, respectively; $p < 0.05$).

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Among specific environmental concerns, waste accumulation and sea lice were cited most frequently (42% each), while escaped farmed fish (18%) and feed sourcing (14%) were considered less significant. These concerns differed from national priorities, where the use of wild fish in feed was more prominent (30%).

Local ownership was strongly favoured: 85% of Connemara respondents preferred locally owned fish farms, compared to 75% in Mulroy Bay and 56% nationally. Similarly, 78% believed coastal planning should be led by local authorities, with only 15% favouring national control.

Support for complementary marine activities was also high: 92% supported marine renewable energy, 88% supported seaweed harvesting, and 77% supported sea fisheries. Seabed mining, however, faced strong opposition (71%), particularly in areas with recent mining license proposals. These findings suggest opportunities for integrated multi-trophic aquaculture (IMTA) and broader marine planning strategies.

**CONCLUSION**

This study highlights how public attitudes toward aquaculture are shaped by proximity and experience. Communities with long-term exposure to fish farming—such as Carna—exhibit more positive perceptions, supporting the existence of a "distance decay" effect. Conversely, communities more removed from aquaculture operations express greater environmental concern.

Tailoring aquaculture development strategies to specific regional and demographic contexts is essential. The influence of age, education, and economic priorities underscores the need for targeted stakeholder engagement and communication strategies.

The strong emphasis on employment benefits reflects the socioeconomic realities of rural coastal communities. Policies that promote local ownership and emphasise local job creation are likely to enhance public acceptance. Broad support for multiple marine activities also points to the potential for integrated marine spatial planning approaches.

To improve MSP implementation, policymakers should:

1. Engage directly with affected communities.
2. Address environmental concerns transparently through monitoring and mitigation.
3. Emphasise local employment benefits.
4. Promote local ownership of marine enterprises.
5. Explore integrated aquaculture systems such as IMTA.

# DRIVING SUSTAINABLE AQUACULTURE THROUGH KNOWLEDGE AND INNOVATION

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Over the last few decades, there has been an urgent need to increase knowledge in the blue economy to promote sustainability and enhance the literacy of employees working in marine areas. Education and training are key drivers for developing and maintaining the skilled workforce required to meet the global challenge of achieving a more sustainable aquaculture industry. Investing in capacity building in aquaculture, marine sciences, and the blue economy is essential for promoting social inclusion and enhancing the competitiveness of the aquaculture sector across Europe.

S2AQUAcoLAB is a private, non-profit institution dedicated to research and experimental development in aquaculture. Its mission is to address the needs of the industry by promoting a smarter and more sustainable aquaculture, grounded in scientific knowledge, innovation, and technological development. One of S2AQUAcoLAB's key priorities is the provision of training programs tailored to the specific needs of the aquaculture sector.

In close collaboration with partners and stakeholders, S2AQUAcoLAB develops technical training programs tailored to the real needs of the aquaculture sector, ensuring industry alignment and immediate applicability. The training offer covers both specialized technical skills — such as marine organism cultivation, aquaculture biotechnology, broodstock management, nutrition, and recirculating aquaculture systems (RAS) — and essential transversal skills. Organized into Essential, Tailored, and Innovation programs, these courses are delivered by experts with hands-on experience and combine face-to-face sessions with online modules via the S2AQUAcoLAB Moodle platform. The goal is to empower professionals, enhance the sector's competitiveness and sustainability, and promote knowledge transfer and technological innovation.

All courses are hosted on S2AQUAcoLAB's Moodle-based online platform, enabling replicability across different organizations, regions, and countries. This digital format offers a strategic opportunity to increase access to training throughout Europe, particularly in coastal and peripheral regions where training in aquaculture and marine sciences may still be limited.

By leveraging its multidisciplinary team's extensive expertise, S2AQUAcoLAB aims to support the competitiveness and innovation of the European aquaculture sector, foster social and territorial inclusion, and contribute to the transition toward a resilient, smart, and sustainable blue economy across Europe.

This project is financed by Missão Interface, project co-funded by PRR - Plano de Recuperação e Resiliência pela União Europeia (01/C05-i02/2022.P148).

# VALORISATION POTENTIAL OF MARINE BY-PRODUCTS IN PORTUGAL: FINDINGS FROM THE AQUAFISH 0.0 PROJECT

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Valorising marine by-products is essential to reducing environmental impacts, enhancing resource efficiency, and strengthening the economic resilience of fisheries and aquaculture sectors. By transforming materials traditionally considered waste into valuable inputs for industries such as aquafeed, pet food, nutraceuticals, and cosmetics, it is possible to advance circular economy practices, open new market opportunities, and reinforce the blue economy value chain, while contributing to biodiversity conservation and aligning with international sustainability targets, including the UN Sustainable Development Goals. In recent years, research in this field has gained momentum due to limited marine resources and growing demand for sustainable sources of functional, nutritional ingredients, and biomaterials. Two main valorisation pathways have emerged: the large-scale production of fishmeal and fish oil for feed, and the development of high-value bioactive compounds using advanced technologies.

Within this context, the AQUAFISH 0.0 project (Interreg Atlantic Area Program) aims to improve the sustainable management and circular use of biomass from fisheries and aquaculture across the Atlantic Area. By fostering collaboration among partners from different regions, the project seeks to identify, quantify, and promote the reuse of by-products and bycatch, driving innovation and reducing waste.

As part of this initiative, an inventory of biomass volumes from by-products and bycatch (category 3, Regulation (EC) No 1069/2009) was undertaken for Portugal. The study applied a combination of direct methods (industry questionnaires) and indirect methods (official statistics, bibliographic sources, and conversion coefficients). Significant data gaps were identified, particularly for aquaculture and fish processing industry by-products, compounded by low response rates from stakeholders. Based on available 2022 production data, marine by-products were estimated at ~41,778 tonnes/year, with the main contributors being the canning industry and frozen fish industry.

A national focus group with sector stakeholders identified key challenges, including the need for refrigeration, storage constraints, and a reduction in the number of processing companies. Participants agreed that the volumes produced are sufficient to support the development of new value-added products, highlighting opportunities for innovation and reinforcing circular economy practices in the Atlantic Area.

This project is financed by “AQUAFISH 0.0: Aquaculture & Fisheries 0.0 – improving the acceptance and social awareness in the consumption of sustainable marine food products developed under the zero-wate philosophy” project co-funded by the Interreg Atlantic Area Programme through the European Regional Development Fund (ERDF) (EAPA_0062/2022).

# EFFECT OF DIFFERENT STOCKING DENSITIES ON CLOSED CAGE HYDRODYNAMICS, WATER QUALITY, FISH HEALTH AND WELFARE

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## Introduction

Closed cage farming has the possible to solve the most important environmental challenges in farming of Atlantic salmon. The most immediate benefit is effective prevention of sea lice infestation, thus also preventing the use of harmful delousing methods and negative effects of salmon lice on wild salmonid populations. Lower risk of fish escapees and the possibility to collect and reuse particular organic effluents may also contribute to improved sustainability in salmon farming. The closed systems also show good growth rates, low feed conversation rates and low mortality (Nilsen et al., 2020). Closed-cage farming enables tighter control of many factors such as water oxygenation level, temperature and hydrodynamic flow. It also allows for closer monitoring of fish health. However, little knowledge exists about how different production intensities in such closed cage systems affect water quality, fish behaviour, fish health and welfare and the final production economy.

## Material and methods

In the project BIOCLOSED we have performed a study at a commercial producer of Atlantic salmon in closed cages, where cages have been monitored over a production cycle. The study includes measurements of fish behaviour, water flow and water quality, studies of the development in water microbiome, registration of fish welfare indicators and analysis of key biological and financial data. The main aim is to investigate the effect of two different initial stocking densities on cage hydrodynamic, water quality, fish health and welfare, and to evaluate the economic output during commercial production with such different stocking densities. The project is organized as a cooperation between one Norwegian industrial partner and the research institutions SINTEF OCEAN, the Norwegian Veterinary Institute and the Norwegian University of Life Sciences.

## Results

The monitoring at the commercial producer will end in May 2025 and the final results will be ready before the conference.

## Litterature

Nilsen, A., Nielsen, K.V., Bergheim, A., 2020. A closer look at closed cages: Growth and mortality rates during production of post-smolt Atlantic salmon in marine closed confinement systems. Aquaculture Engineering, 91, 102124.

# IMPACT OF METHIONINE SUPPLEMENTATION ON IMMUNE REGULATION AND RESISTANCE OF ATLANTIC SALMON (*Salmo salar*) TO SALMON LOUSE (*Lepeophtheirus salmonis*)

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Atlantic salmon (*Salmo salar*) farming faces significant challenges due to infestations by *Lepeophtheirus salmonis*, leading to considerable financial losses. Recently, extensive research has focused on the interaction between host and parasite to create effective treatments or preventative strategies against salmon lice. Adding methionine to fish feed has been shown to improve resistance to bacterial infections. Nonetheless, there has been no investigation into the immunomodulatory effects of this amino acid on parasite levels in aquaculture settings. This study sought to assess the impact of dietary methionine supplementation on the immune response of Atlantic salmon when exposed to sea lice. Juvenile fish (approximately 120g) were fed for a period of four weeks with either a standard control diet (CTRL) or a diet supplemented with methionine (MET, at twice the recommended level of L-methionine), and after sampled (n=12 per diet group). The remaining fish were then exposed to sea lice infection through cohabitation (100 copepods per fish) and sampled two weeks following the challenge. Samples of blood, plasma, liver, and head kidney were collected for haematological assessments, analysis of systemic humoral responses, oxidative stress markers, and gene expression studies, respectively. Moreover, skin samples were taken for proteomic analysis and RNA sequencing at both pre-infection and post-infection stages from parasite attachment sites and skin after this local. Parasite counts per fish showed that 70% were at the chalimus-II stage, with the remainder being at the pre-adult stage, and no significant differences were observed between the dietary groups. At the chalimus-II stage, the parasite feeds exclusively on mucus and skin, which could explain the limited haematological response detected, although infection appeared to elevate white blood cell counts. These findings, alongside the absence of oxidative stress biomarkers and immune gene regulation in the head kidney, indicate that while the ectoparasite was present, it did not provoke a notable systemic reaction in the host. This conclusion is further supported by the lack of lipid peroxidation in the tissues. Taken together, this supports the idea that during early development, the parasite primarily triggers immune responses locally at the skin. These responses, largely driven by tissue damage, may not generate a systemic immune reaction. However, plasma protease activity in the MET-fed group rose significantly following infestation compared to both the basal diet group and the uninfected controls. The proteomics and RNA sequencing data from the skin infected and skin where the lice attachment sites are currently under analysis to provide deeper insights into methionine's immunomodulatory potential and the local immune response associated with resistance to salmon louse.

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# AQUACULTURE, A FAVORABLE RECOMMENDATION FOR FOOD INSECURITY, POVERTY AND UNDERNUTRITION IN KENYA

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Food insecurity remains one of the most evident aspects of poverty. The growing population and the competition for resources such as land and water imply that global food demand is burgeoning. In Kenya, food insecurity trends are worrying as the population is predicted to hit 55 million by 2030 as the per capita annual growth rate of arable land and the increase in food prices. Aquaculture is vital for enhancing food security, alleviating poverty and promoting sustainable economic growth in Kenya. The main challenges in the aquaculture subsector in Kenya include; Inadequate availability and affordability of quality fish seed (fingerlings), lack of high quality and affordable fish feeds, inadequate supportive infrastructure e.g. hatcheries, fish marketing systems, fish processing plants, shortfall in budgetary allocations to support aquaculture initiatives, weak research- extension-farmer linkages, slow adoption rate of fish farming technologies, innovations and management practices (TIMPs), lack of good credit facilities and schemes for fish farmers, poor security and safety of fish in ponds and cages, poor book keeping and record management leading to inaccurate data, policy and legal framework for fish feed and seed certification to monitor compliance. The opportunities to enhance aquaculture contribution to food security, poverty alleviation and malnutrition in Kenya include; integration of aquaculture into conventional farming, upscaling modern technologies, innovations, and management practices (TIMPs), competency- based training on special skills in aquaculture, organizing fish traders into groups to maximize financial and market opportunities, exploring opportunities in value- added product development, investment in modern fish processing technologies and marketing techniques, promoting public –private partnerships to support aquaculture initiatives.

# SUITABILITY OF CONVENTIONAL INGREDIENTS USED FOR AQUAFEED PRODUCTION IN KENYA

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The ambitious aquaculture production targets aim at producing 1,000,000 mt, 700,000 mt, 50,000 mt and 160,000 mt in Uganda, Kenya, Tanzania and Rwanda, respectively by 2030;this therefore calls for increased fish feed production however fisheries value chain is challenged by limited access to quality fish feeds that are commercially competitive and environmentally friendly. Small scale fish farmers are limited by high feed cost and logistics associated with feed importation, that limit their access to quality feeds. Accordingly, there is growing interest to promote backyard aquafeed formulation. Traditionally feed formulation focuses on nutrient composition (more so proximate composition) and cost of feed ingredients, and pay less attention to several factors that affect sustainability, including pollution potential, environmental/ecological impacts, competing uses, socio-cultural considerations, yield and fertilizer requirements among others.

We examined a broad range of factors that may affect the suitability of a feed ingredient (including those related to environment, nutrition content and utilization, unit nutrient cost, and socio-economics among others) for five commonly used protein ingredients (i.e., fishmeal, bone meal, soya bean meal, sunflower meal and cotton seed meal), and four conventional energy ingredients (i.e., maize bran, sorghum grain, wheat bran and rice bran) in Kenya. The results indicated that soya bean and cotton seed meals are the most viable plant-based protein ingredients compared to sunflower meal average. Among the animal-based protein ingredients, omena fish meal out performed bone meal. However, continued use of fishmeal in feed formulation seems unsustainable because of the allied ecological and environmental impacts. Maize bran remains the most viable energy ingredient, outperforming wheat bran, rice bran and sorghum, respectively. Overall, animal ingredients are the most suitable protein sources for farmed fish species. However, their use is associated with ecological and environmental impacts that culminate into outstanding negative impacts to the aquatic ecosystem than plant proteins ingredients.

# AQUACULTURE, A PROMISING SOLUTION FOR FOOD INSECURITY POVERTY AND MALNUTRITION IN KENYA

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Food insecurity remains one of the most visible dimensions of poverty. The increasing population amid competition for land and water resources means that the global demand for food will continue to increase. In Kenya, the food insecurity trend is worrying as the population is expected to hit 55 million by 2020 against an annually declining arable land per capita and consequent increase in food prices. The Kenyan agricultural sector has failed to either eliminate or reduce malnourishment for poor populations as the annual national production for both staple food and livestock products fall short of national consumption levels. With increasing food production challenges like dwindling capture fisheries and impacts of climate change becoming more eminent, solutions to food insecurity and malnutrition in Kenya must bring quick results in food availability by stimulating more own-food production. Aquaculture has so far been recognized as an important opportunity to enhance household food security in developing countries. Aquaculture, the controlled land-based or open-ocean farming of aquatic organisms such as tilapia, catfish, finfish, shellfish and plants, is the fastest growing food sector globally alongside terrestrial crop and livestock production. Fish provides protective effects on a wide range on health including obesity, stroke, high blood pressure, and coronary heart disease. Fish has a nutrient profile superior to all terrestrial meats, an excellent source of high quality animal protein, omega-3 polyunsaturated fatty acids (PUFAs) and vitamins. Unfortunately, in Kenya, fish has been only marginally included in the national debate on reduction of micronutrient deficiency, precisely where it could potentially have the largest impact.

# POLYCULTURE IN AN AQUAPONIC SYSTEM : IMPROVING SYSTEM EFFICIENCY BY RAISING REDCLAW CRAYFISH ON PIKEPERCH LEFTOVER FEED

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## Introduction

Interest is growing for aquaponics as a way to reduce environmental negative impact of running aquaculture and hydroponics systems separately. The PolyRAS project (Interreg GR) aims to improve the production of fishes with local interest using polyculture in different intensive systems (RAS, aquaponics, biofloc), in order to increase production while optimizing resources use. Intensive pikeperch (*Sander lucioperca*) aquaculture is considered as a good prospect for the European market due to its fast growth rate and economical expectation. However, pikeperch is very sensitive to environmental stressors (light, noise, …) that can impair feeding behavior, leading to frequent overfeeding. Uneaten feed can be turned from waste into valuable feed resource by introducing an auxiliary species in a polyculture scheme. Redclaw crayfish (*Cherax quadricarinatus*) appeared as a good auxiliary candidate as it is an omnivorous bottom feeder, its rearing requirements are compatible with pikeperch and aquaponics, and crayfish are a valuable product on the European market.

The aim of the work was to assess if the efficiency of an intensive aquaponic pilot system can be improved by rearing redclaw crayfish on uneaten pikeperch feed, thus improving productivity while reducing environmental impacts by sharing the same space, water and feed resources.

## Material and Methods

This polyculture combination (pikeperch and redclaw crayfish) was reared in a coupled aquaponic pilot facility to assess the efficiency of the system. The aquaponic facility has a surface of 100 $m^2$, and a total volume of 18 $m^3$. It is composed of 6 rearing tanks (total volume: 9 $m^3$) and a crop production area of 50 $m^2$. Temperature was set at 24°C, mean pH and oxygen levels were 6,8 ± 0,1 and 7,7 ± 0,3 mg/L respectively and mean nitrates concentration ($NO_3^-$) was 492 ± 90 mg/l.

At the start of the experiment, pikeperch stoking densities, ranged from 22 to 30 kg/$m^3$ depending on the size of fish. Crayfishes were placed at the bottom of pikeperch rearing tanks, protected by cages to avoid any predation risk. Crayfish were stocked at 6 individuals/$m^2$ to avoid territorial aggressive behavior. Cages were stocked with animals similar in size, and of the same sex to reduce aggressiveness and avoid reproduction. Moreover, PVC pipes were provided as shelters.

Pikeperch were fed using automatic feeders (LINN Profi) distributing Le Gouessant percid feed for a daily 12h period. Crayfish were only fed on leftover food from the pikeperch for the duration of the experiment.

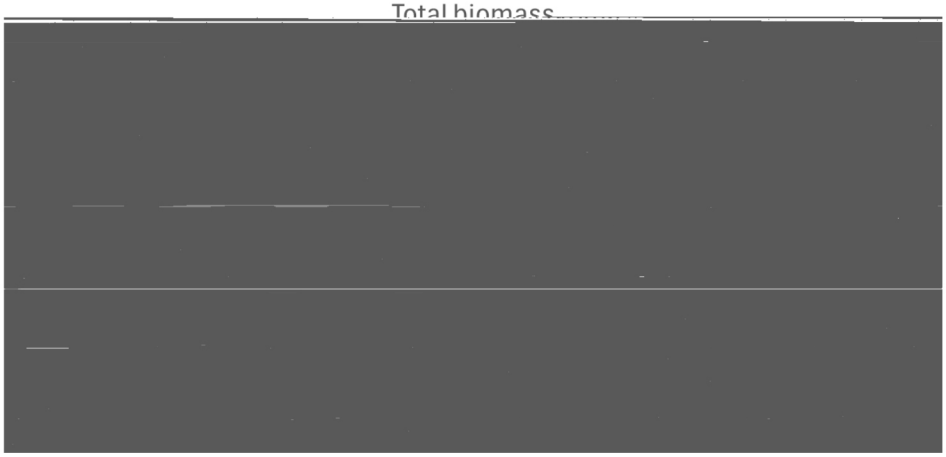


*Figure 1. Number of crayfish and total biomass during the four-month production period.*

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The vegetal production consisted in lettuce and different aromatic herbs (parsley, basil, cilantro and arugula). System productivity as well as fish welfare and waste production will be assessed on a 6-month period. Pikeperch welfare will be evaluated by measuring cortisol in scales. Crayfish welfare will be assessed using claw integrity as an indicator. Drumfilter functioning and sludge release will be analyzed to assess solid waste production and environmental impacts.

**Preliminary results and discussion**

Pikeperch production reached 438 kg/yr and survival rate was 94%. Global FCR was 1,1 and specific growth rates ranged from 0,5 to 1,2 depending on fish size. Those results are similar to pikeperch monoculture production in the same system. Crayfish growth was positive in all tanks and global crayfish production reached 1,9 kg/yr with a survival rate of 70%.

Reduced crayfish survival rate is still under investigation. Water parameters stayed in the acceptable range for crayfish production and cannibalism could not be the cause as suggested by the good claw integrity level.

Polyculture between pikeperch and redclaw crayfish seems promising to improve system efficiency by producing biomass on leftover feed. The next step will be to assess the impact of this combination on waste management and animal welfare.

# COUPLED BIOMETRIC AND PHYSIOLOGICAL INDICATORS REVEAL THE IMPACTS OF CLIMATE CHANGE–DRIVEN ACIDIFICATION ON THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*)

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## Introduction

Human activities have already caused a decrease in the pH of the oceans by approximately 0.1 units, with an average decline of about 0.02 units per decade (Doney et al., 2009). The North Adriatic basin experiences acidification rates that exceed global averages due to river input which intensifies pH fluctuations (Cantoni et al., 2024). Mytilus galloprovincialis, a dominant species in coastal areas responsible for over 60 percent of Italian mussel production, plays a crucial role in the ecosystem through water filtration, nutrient removal, and habitat creation. However, the biomineralization of its shel depends on carbonate saturation and is highly sensitive to decreases in pH (Waldbusser et al., 2015). Reductions in pH can impair linear growth, weight gain, and filtration rate (Bressan et al., 2014). These combined sub-lethal effects undermine the o fitness and commercial value of mussels, making the northern Adriatic a critical area for evaluating the vulnerability of calcifying organisms. Integrated analyses of biometric, and physiological parameters help to address existing gaps in understanding the species' adaptability. The understanding the resilience thresholds of M. galloprovincialis is vital for supporting adaptive management strategies in the climate scenarios projected for the 21st century.

## Material and Methods

Juvenile Medietrranean mussel (*Mytilus galloprovincialis*) were collected from the North Adriatic Sea, cleaned, and acclimated for one week at 24 °C, salinity 27 ‰ and pH 8.2. After tagging and removal of byssal threads, mussels were distributed by weight into 12×70 L aquaria (≈ 25 individuals each). Four pH were imposed—ambient (8.2) plus three acidified levels (7.9, 7.7, and 7.4)—with $CO_2$ bubbling used to reach and maintain target values; each treatment had three replicate tanks. All systems were mechanically and UV-filtered continuously aerated and fed daily with a mixed-algal ration equal to 5–6 % of mussel wet mass. The experiment ran for 90 days, a period expected to allow roughly 1 cm of shell growth to the commercial size of ~4 cm. Temperature, salinity, dissolved oxygen, and pH were monitored daily; total alkalinity was measured weekly thereafter to calculate $pCO_2$, and full carbonate chemistry. Mortality was recorded daily. At day 0, day 45, and day 90, subsamples of ten mussels per treatment were analyzed for shell length, thickness, total wet and dry mass, meat yield, condition index, hepatopancreas mass, and specific growth rate. The clearance rate was quantified at the beginning and end of the trial on three mussels per treatment by tracking the exponential decline of algal cells in 1 L beaker over 2 h, with samples taken every 30 min for microscopic counts.

## Results

At the onset of the experiment shell length did not differ among pH treatments (8.2, 7.9, 7.7, and 7.4). A one-way ANOVA on 250 tagged mussels (n = 62–63 per pH) yielded $F_{3}, 246 = 1.08$, $p = 0.36$, confirming homogeneity of baseline size. Mean length increased steadily at all pH levels. Linear mixed-effects modeling with a random intercept for each individual detected a strong main effect of Time ($p < 0.001$) but neither a main effect of pH ($p \geq 0.07$) nor a pH × Time interaction (all interaction terms $p > 0.10$). Growth therefore followed parallel trajectories irrespective of carbonate chemistry. Mussels elongated by 2.1–2.5 mm, equivalent to 0.043–0.051 mm $day^{-1}$. Shorter windows showed slightly faster daily rates during the first 18 days (0.07 mm $day^{-1}$) than during the subsequent days (0.05 mm $day^{-1}$), but the pattern was consistent across treatments. The data suggest a trend toward faster clearance at the optimal. pH Single-vessel time-courses of algal concentration fitted well to first-order exponential decay. The derived rate constants (k) translated to volumetric clearance rates of 0.28 ml $min^{-1}$ (16.8 ml $h^{-1}$) at pH 7.7, 0.27 ml $min^{-1}$ (16.1 ml $h^{-1}$) at pH 7.4, and 0.41 ml $min^{-1}$ (24.5 ml $h^{-1}$) at pH 8.2.

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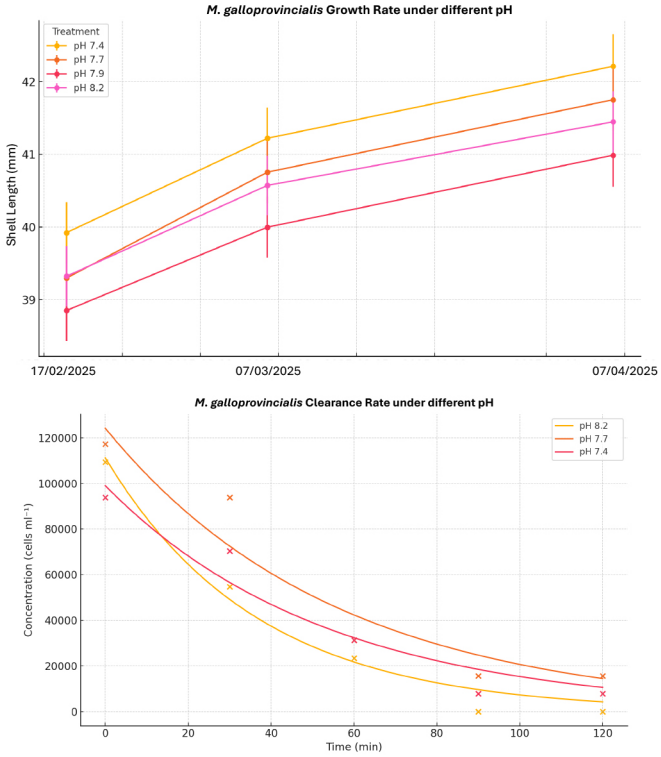


## Discussion

Over seven weeks, the animals grew at identical rates (~0.05 mm day$^{-1}$, ≈ 2.3 mm total) across seawater pH 8.2–7.4, indicating short-term shell-length growth is insensitive acidification. Yet feeding assays revealed a ~35 % drop in clearance rate below ambient pH, suggesting energy acquisition is already compromised. This divergence implies mussels can initially maintain shell extension by reallocating resources, but longer or multi-stress exposures may reveal hidden costs. Thus, while early growth appears resilient to near-future ocean acidification, reduced feeding efficiency emerges as an early warning signal.

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# AQUALOOP CROSS-BORDER STUDENT EXCHANGE PROGRAM: PROMOTING CIRCULAR AQUACULTURE THROUGH INTERNATIONAL COLLABORATION

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## Introduction

The AquaLoop Cross-Border Student Exchange Program, held between January and June 2025, aimed to foster international collaboration and practical knowledge transfer in the field of sustainable aquaculture and circular economy. Organized within the AquaLoop - Aquaculture expert floor for circular economy practice project framework, the program involved bachelor's and master's students from the University of Rostock (Germany), University of Gdańsk (Poland), and Klaipeda University (Lithuania).

Through week-long visits at partner institutions, students were immersed in hands-on activities centered on the AquaLoop project's three pilot actions:

- TARAS: Testing Algae Applications in Recirculating aquaculture systems (RAS) to improve aquaculture circularity potential in the SB region
- NEMATIC: Increasing the nutrient efficiency of commercial aquaculture through increased application of circular economy concepts
- FISHVISA: Development of the Fish-Shrimp-Vegetables integrated system of aquaponics to showcase the potential of circular economy principles in RAS

## Methods

Each university hosted short-term (one week long) exchange visits that included:

- Laboratory and field-based learning: Participants conducted experiments and aquaculture routines based on AquaLoop pilot studies.
- Varied thematic focus:
    - University of Gdańsk: Microalgae cultivation and shrimp farming in RAS (TARAS)
    - University of Rostock: Fish nutrition, aquaponics, and fish leather production (NEMATIC)
    - Klaipeda University: Integrated aquaponics and multitrophic systems (FISHVISA)
- Field visits: Participants visited commercial aquaculture facilities such as:
    - K2 Trout Farm (Poland)
    - Institute for Fisheries at the State Research Centre Mecklenburg-Vorpommern in Hohen Wangelin (Germany)
    - Martynas Klevinis Arctic Char Farm (Lithuania)
- Post-visit surveys to evaluate participants' satisfaction and learning outcomes
- Certificates of attendance

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**Results**

The program successfully engaged 19 students in structured exchange weeks across the three countries. Key outcomes include:

- Practical skills development in microalgae cultivation, shrimp and fish RAS operations, fish, shrimps, and crayfish feeding trials, aquaponic plant care, and advanced laboratory methods such as parasitology and molecular fish tissue analysis.
- Enhanced students' understanding of circular practices in aquaculture, strengthened cross-border networks, and inspiration for future innovation in sustainable aquatic food production.
- Enhanced cooperation between industry and academia fostered through study visits during exchange stays, providing students with valuable career development opportunities and exposure to real-world challenges in aquaculture.
- High satisfaction across all exchanges. Evaluation scores ranged from 90% to 100% in categories such as communication, organization, collaboration, and overall satisfaction.
- Networking and cultural exchange. Students valued cross-border peer collaboration, sightseeing, and informal discussions with faculty and fellow participants.
- Further collaboration between project partners and their students in the scientific field, fully aligned with AquaLoop's objectives of promoting knowledge exchange and the development of sustainable aquaculture technologies through international collaboration.

**Conclusion**

The AquaLoop Cross-Border Student Exchange Program demonstrated a highly effective model for international, practice-based learning in aquaculture and circular economy. By combining experimental training, interdisciplinary collaboration, and regional aquaculture insights, the program enriched academic curricula and helped shape the next generation of sustainable aquaculture professionals. The strong positive feedback confirms the program's value and provides a foundation for future exchanges in the South Baltic region and beyond.

# IMPROVING THE CIRCULARITY OF AQUACULTURE IN THE SOUTH BALTIC REGION – A PILOT STUDY ON THE USE OF MICROALGAE IN RAS SYSTEMS WITH WHITELEG SHRIMP *Penaeus vannamei*

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## Introduction

Due to climatic conditions and the lack of permanent access to warm seawater, the production of whiteleg shrimp *Penaeus vannamei* in most of Europe is practically only feasible in land-based recirculating aquaculture systems (RAS). Although such systems allow for significant waste reduction by recycling and reusing water, they are not 100% efficient, especially at high stocking densities. Some of the water in the RAS must therefore be regularly replaced. Thus, there is ongoing interest in research focused on developing more effective circular economy solutions for RAS systems which aim not only to minimize waste but also to generate additional value. One promising approach is the cultivation of microalgae, which purify nutrient-rich effluent from a recirculating water system (RAS) during growth and, in addition, produce a biomass rich in omega-3 fatty acids that can serve as a dietary supplement for shrimp. The aim of our pilot study was to identify Baltic microalgae strains able to grow in wastewater from whiteleg shrimp RAS aquaculture, while producing a high yield of biomass.

## Methods

The first step of the research was the preparation of a small-scale laboratory RAS system, consisting of a main unit (equipped with, among other components, a water purification and parameter control system) and four tanks containing water for shrimp cultivation with a total capacity of 1200 L. The next step was importing *P. vannamei* post-larvae (n=1000) from a hatchery in Austria (White Panther Produktion GmbH) and continuing their cultivation at a temperature of 27°C, salinity of 30 ppt, pH ~7.8 and a photoperiod of 15 h of light and 9 h of darkness. The shrimps were fed five times a day with commercial feed, with the daily portion adjusted according to their biomass.

In the 13th week of shrimp farming, 10 L of wastewater was collected, and the concentrations of ammonium nitrogen ($N\text{-}NH_3^+$), nitrates ($N\text{-}NO_3^-$), phosphates ($PO_4^{3-}$) and silica ($SiO_2$) were determined chemically using colorimetric cuvette tests (Hach Lange GmbH, Germany). The water was high in nitrates (122 mg $L^{-1}$), whereas the concentration of other nutrients was low. For this reason the wastewater required certain modifications to achieve a favorable N:P (24:1) as well as to increase the concentration of silica and iron. Microalgae culture was carried out in two media: undiluted wastewater (30 ppt, control) and diluted with Baltic seawater (18 ppt), because many Baltic microalgae are brackish rather than marine ones.

For the tests, three strains of diatoms were chosen, i.e., *Amphora* sp. CP3, *Gedaniella* sp. PL1.21 and *Nitzschia* cf. *aurariae* BA158. The diatoms were grown in the modified wastewater for 12 days. Their growth was monitored every 2 days by measuring their optical density (OD) at 750 nm, which was later converted to cell density. At the end of the culturing period, the concentrations of photosynthetic pigment and biochemical composition of the biomass were determined. Finally, nutrient concentrations were measured to estimate the efficiency of nitrate and phosphate removal.

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## Results

All of the tested strains actively grew in the RAS wastewater. The best growth was observed for *N.* cf. *aurariae* BA158 in the diluted RAS water supplemented with increased silica and iron amounts (ca. 400 mg $L^{-1}$), which was comparable to the control culture (i.e., f/2 medium, salinity 30 ppt). The same culture also showed the highest production of lipids (70 mg $L^{-1}$) and fucoxanthin (0.75 mg $L^{-1}$). All strains removed a significant proportion of nutrients, however, the most efficient strain was *Amphora* sp. which removed 77-85% of nitrates and 80% of phosphates.

## Conclusion

The results of this pilot study showed that the RAS wastewater can be used as a medium for the efficient growth of Baltic Sea microalgae to remove excess nutrient concentrations. However, the success of this approach depends on appropriate modification of the wastewater to achieve the correct N:P ratio and, if necessary, to adjust silica and iron concentrations. Since all of the strains efficiently removed nutrients, the choice of the strain for cultivation should be based on its ecophysiological and biochemical traits to produce high-quality and commercially viable biomass, thereby adding value to RAS systems.

## Acknowledgements

The research was carried out as part of the AquaLoop INTERREG SB project.

# EFFECT OF ANAEROBIC DENITRIFICATION IN *Penaeus vannamei* PRODUCTION UNDER BIOFLOC SYSTEM

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## Introduction

Anaerobic denitrification consists of the microbial conversion of nitrate and nitrite into molecular nitrogen or nitrous oxide, promoting the removal of these compounds from aquatic environments. In aquaculture, this process improves water quality, prevents eutrophication, and reduces dependence on chemical inputs, thus promoting sustainability. This work aimed to test different strategies to optimize anaerobic denitrification in biofloc systems during the cultivation of *Penaeus vannamei*, evaluating different denitrification volumes and different concentrations of suspended solids.

## Materials and methods

A preliminary three-week trial was conducted to assess the potential denitrification capacity of biofloc. Initially, biofloc was concentrated by decantation to levels of 3000 and 4000 mg/L and then exposed to water with a high nitrate concentration (approximately 305 mg N-$NO_3$/L). Since denitrification requires anaerobic conditions, molasses was added to achieve a carbon-to-nitrogen (C:N) ratio of 3:1. The pH was consistently maintained above 8.0 by adding a 1 M sodium hydroxide (NaOH) solution. Dissolved oxygen, temperature, and pH were monitored hourly for 12 hours during the denitrification process until stabilization. The process was considered complete when nitrate concentrations approached 0.0 mg/L. Chemical analysis of ammonia nitrogen (TAN), nitrite ($NO_2^-$, mg/L), nitrate ($NO_3$-, mg/L), phosphate levels ($PO_4^{3-}$, mg/L), alkalinity (mg/L) were analyzed. To evaluate the impact of exchanging 25% of denitrified water in *Penaeus vannamei* production under biofloc technology (BFT), six 350-L tanks stocked at a density of 150–200 shrimp/m² (will be divided into two experimental groups: a control group and a denitrification group, with the latter receiving two weekly water exchanges of 20% of the tank volume using denitrified water.

## Results

Along the progressive scaling of denitrification tanks, the total nitrate was reduced to atmospheric $N_2$ after 48 hours of denitrification process (Figure 1).

Key findings indicate that the higher concentration of suspended solids (4000 mg/L) exhibited nitrate removal efficiency comparable to the lower concentration (3000 mg/L), with minimal differences in stabilization parameters such as pH, DO, and alkalinity.

## Discussion

In the present study, nitrate levels dropped from 341.845 mg/L to 0 mg/L within 48 hours, indicating complete removal. This aligns with Silva (2025), where denitrification occurred within 24 hours in treatments with 2000–2500 mg/L of suspended solids. However, our study required more time, reflecting system variations. The biofloc system effectively maintained low ammonia and nitrite levels while achieving nitrate removal.

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**Acknowledgments**

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# DENSITY-DEPENDENT MODULATION OF SPERM MOTILITY: A REGULATORY MECHANISM WITH CONSEQUENCES FOR MEASUREMENTS STANDARDISATION AND SPERM COMPETITION

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## Introduction

Sperm motility is a key determinant of fertilization success in externally fertilizing fishes. While dilution is inevitable through activation, its effects on motility are not fully understood. In various fish species, we observed a paradoxical pattern: increasing sperm concentration was associated with reduced sperm velocity. This raised critical questions about the biological underpinnings of sperm behaviour and the reliability of motility measurements under varying conditions. We therefore experimentally tested the effect of cell density on sperm motility.

## Materials and Methods

Semen samples were collected from 12 Eurasian perch *Perca fluviatilis* and measured after activation under 5 different dilution ratios (1:25 to 1:200), under constant or variable osmolarity (i.e. 60 treatments), to disentangle effects from cell density and osmolarity variation (typical with dilution). To disentangle potential physical (cell collisions) and biological effects, we conducted an experiment comparing velocity patterns of the same samples in controlled proportion (0, 12.5, 25, 50 and 100%) of live and dead sperm. Motility parameters were quantified using Qualisperm CASA on a total of 180 scenario. Linear mixed-effects models were used to assess the effects of dilution, osmolarity, and live/dead ratio on sperm velocity and progressive motility.

## Results

Sperm motility and dilution were inversely correlated regardless of osmolarity. When looking closer, we found that it was due to the activation of slow sperm (5-25 $\mu$m/s) at lower sperm dilution / higher cell densities. Indeed, neither fast sperm (> 25 $\mu$m/s) nor non progressive sperms (1-5 $\mu$m/s) proportions were affected by dilution. The live-dead sperm experiment revealed that at constant cell density, the velocity was significantly negatively affected by the proportion of live sperm. Together, these results suggest an active, biologically mediated mechanism rather than passive physical interference, which modulates activation of slower sperm in the context of higher live cell densities.

## Discussion

These findings challenge the assumption that sperm motility measurements are concentration-independent and call into question the comparability of published data without standardized protocols. Our results highlight the importance of live-cell-dependent density effects, possibly involving chemical signalling, ionic feedback, or metabolic interactions among spermatozoa. Such mechanisms may reflect evolved strategies of sperm competition, where motility is modulated in response to perceived gametic density. These insights have direct relevance for artificial fertilization practices in aquaculture and offer a new lens for understanding reproductive strategies in externally fertilizing species.

# DL-METHIONINE FORTIFIED AVOCADO SEED MEAL REPLACED 50% OF SOYBEAN MEAL IN THE DIETS OF AFRICAN CATFISH, *Clarias gariepinus*

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## Introduction

Avocado pear (*Persea americana,* Mill) is a widely consumed fruit because of its delicious taste, rich nutrient composition and several-health promoting bioactive ingredients. It is rich in minerals, vitamins and protein. However, the seed which is discarded as a waste makes up between 15 and 17% of the whole fruit. Preliminary analysis of the seed used in the study showed high protein level of 42.5% which is closely related to the protein level of 45% in soybean meal. Hence the study investigated the effect of total replacement of soybean meal with the Avocado sees meal (AM) in the diets of African catfish. DL-methionine was added due to its limitations in plant protein feed ingredients.

## Materials and methods

Five isonitrogenous diets were prepared to contain 0, 25, 50, 75, 100% of Avocado seed meal (AM) in replacement for soybean meal in making diets 1 -5. Then diets 1-5 were fortified with 0, 0.20, 0.40, 0.80, 1.0 % DL-Met respectively. The diets were fed to African catfish (10.2 g) for 56 days in indoor glass tanks. Standard procedures were used in data collection and analyses.

## Results

Analysis of the Avocado seed meal used in the study revealed a high protein content of 42.5% and high fibre and lipid contents (Table 1). Assessment of the mean weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio indicated that AM can replace up to 50% of soybean meal in the diet of the fish (Table 2). Similarly, dietary AM significantly improved Mg, Ca, P, Mn and Zn deposition in the fish in comparison with the control diet containing 100% soybean meal (Table 3). Moreover, dietary AM significantly reduced the glucose levels in the experimental fish (Fig. 1).

## Conclusion

Replacement of soybean meal with Avocado seed meal at (50% +0.4% Met) is recommended for the production of African catfish. Replacement of soybean meal with AM also significantly improved the minerals and reduced the carcass glucose levels.

Proximate composition of the Avocado seed meal (AM)

| Parameters | Values |
|---|---|
| Crude protein | 42.5 |
| Ash content | 1.00 |
| Lipid | 13.6 |
| Moisture | 9.00 |
| Crude fibre | 33.4 |
| NFE | 0.50 |

*(Continued on next page)*

Table 2. Growth performance of African catfish fed methionine fortified Avocado seed meal in replacement for Soybean meal

| Parameters | Diet 1 0 % AM | Diet 2 25% AM | Diet 3 50% AM | Diet 4 75% AM | Diet 5 100% AM |
|---|---|---|---|---|---|
| Initial Wt. (g) | 10.3 | 10.2 | 10.2 | 10.2 | 10.1 |
| Final Wt. (g) | $51.8^{cd}$ | $52.7^{cd}$ | $47.8^{c}$ | $40.9^{b}$ | $29.1^{a}$ |
| Weight gain | $50.1^{cd}$ | $52.7^{cd}$ | $47.8^{c}$ | $40.9^{b}$ | $29.4^{a}$ |
| SGR | $1.26^{cd}$ | $1.26^{cd}$ | $1.19^{c}$ | $1.02^{b}$ | $0.84^{a}$ |
| FCR | $1.60^{a}$ | $1.56^{a}$ | $1.74^{ab}$ | $1.95^{c}$ | $2.41^{d}$ |
| PER | $1.45^{cd}$ | $1.48^{cd}$ | $1.33^{c}$ | $1.25^{b}$ | $1.00^{a}$ |

Table 3. Mineral contents of African catfish fed methionine fortified Avocado seed meal in replacement for Soybean meal (mg/g)

| Parameters | Diet 1 0 % AM | Diet 2 25% AM | Diet 3 50% AM | Diet 4 75% AM | Diet 5 100% AM |
|---|---|---|---|---|---|
| Mg | $18.7^{a}$ | $21.9^{b}$ | $26.0^{c}$ | $20.6^{b}$ | $17.1^{a}$ |
| Ca | $25.8^{a}$ | $30.9^{c}$ | $31.7^{c}$ | $27.2^{ab}$ | $27.7^{ab}$ |
| P | $7.24^{a}$ | $9.96^{b}$ | $9.13^{b}$ | $9.44^{b}$ | $9.12^{b}$ |
| Mn | $0.09^{b}$ | $0.11^{c}$ | $0.11^{c}$ | $0.08^{a}$ | $0.92^{b}$ |
| Zn | $0.77^{a}$ | $1.13^{c}$ | $1.05^{c}$ | $0.84^{b}$ | $0.88^{b}$ |

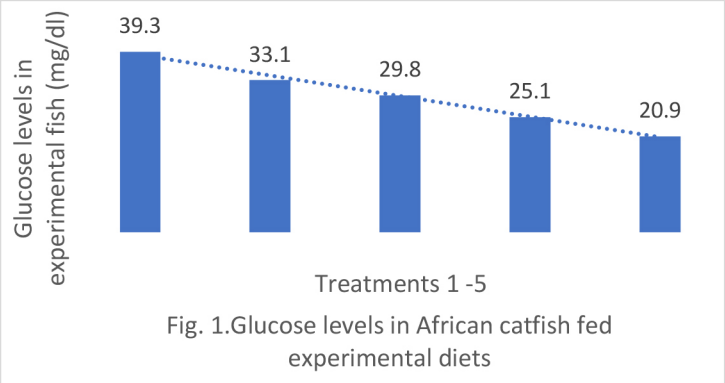


Fig. 1.Glucose levels in African catfish fed experimental diets

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# DEVELOPING A VACCINE AGAINST *Aeromonas veronii*, AN EMERGING PATHOGEN IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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## Introduction

*Aeromonas veronii* is a Gram-negative bacterium that poses a significant threat to sea bass aquaculture in the Mediterranean. This pathogen causes severe systemic infections, leading to high morbidity and mortality rates among farmed sea bass. The economic impact of these infections is substantial, affecting the profitability and sustainability of sea bass farming in the region.

## Methods and Results

Field isolates of *A. veronii*, including both motile and non-motile strains, were collected from commercial sea bass farms in the Mediterranean. These isolates were characterized using a combination of biochemical methods, genotypic techniques (such as metG and gltA sequencing), and serological assays, providing valuable insights into their differences.

Based on these characterizations, experimental vaccines were developed and tested in pre-clinical laboratory trials. The trials involved vaccinating and challenging sea bass with different strains and recording clinical signs and mortality rates. The experimental vaccine candidates demonstrated rapid and effective protection against *A. veronii*, showing promising results for long-term immunity.

## Conclusion

Developing an effective and long-lasting vaccine against *A. veronii* is essential for the sustainability of sea bass farming in the Mediterranean. Our pre-clinical laboratory trials have yielded promising results, identifying vaccine candidates that provide both rapid and extended protection against *A. veronii*. These findings highlight the potential of a future vaccine to significantly reduce the impact of *A. veronii* infections on sea bass aquaculture.

The pre-clinical studies were conducted in line with the “License to Carry out Research on Animals” granted to AquaBioTech Group by the Ministry for Agriculture, Fisheries, Food and animal rights, Animal welfare Department in Malta and in accordance with procedures approved by the Zoetis Animal Ethics Committee.

# ENABLING PRODUCTION OF THE RARE CAROTENOID DIATOXANTHIN AT INDUSTRIAL LEVEL USING CRISPR/CAS9 GENE-EDITED MICROALGAE

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## Introduction

Recent research has indicated that the rare carotenoid diatoxanthin (Dtx) exhibits higher bioactivity than commercially available pigments, and that it has anti-inflammatory and anti-cancer properties (Konishi et al., 2008; Pistelli et al., 2021; Sansone et al., 2025, 2023, 2022). These findings highlight Dtx as a new marine compound of commercial interest. Dtx is only found in a few groups of microalgae where it is produced as protection against exposure to sudden high light intensity, and it can be viewed as a "sunscreen" pigment (Goss and Lepetit, 2015). Large-scale commercial production of this pigment from microalgae is currently not feasible. In fact, Dtx is rapidly converted to the inactive pigment diadinoxanthin (Ddx) when the cells are removed from the high light source (Goss and Lepetit, 2015; Græsholt et al., 2024), which will be the case during large-scale harvesting of algal biomass. In addition, producing commercially interesting levels of Dtx in "natural" microalgae will depend on exposing the algae culture to high intensity light for a prolonged period of time. This can be technically challenging at high cell densities and costly in countries where algae production is dependent on artificial light. Using the gene editing technique CRISPR/Cas9 we have created non-transgenic *zep3Cpsrp54* double knockout (KO) mutants of the marine diatom *Phaeodactylum tricornutum*. The *ZEP3* gene encodes zeaxanthin epoxidase responsible for back-converting high light-accumulated Dtx to Ddx in low light, whereas the *CpSRP54* gene encodes the chloroplast signal recognition particle 54 kDa (CpSRP54) protein involved in insertion of subunits of photosynthetic complexes into thylakoid membranes (Græsholt et al., 2024; Nymark et al., 2021). We have previously shown that loss of ZEP3 can stabilize high light-accumulated Dtx, whereas absence of CpSRP54 causes light sensitivity (Græsholt et al., 2024; Nymark et al., 2021). The loss of both ZEP3 and CpSRP54 in *P. tricornutum* was hypothesised to result in a Dtx production line where both the above-described challenges would be solved. Producing a high-value product like Dtx can cause microalgae cultivation to become economically viable, opening the possibility of using the rest of the algal biomass for feed purposes.

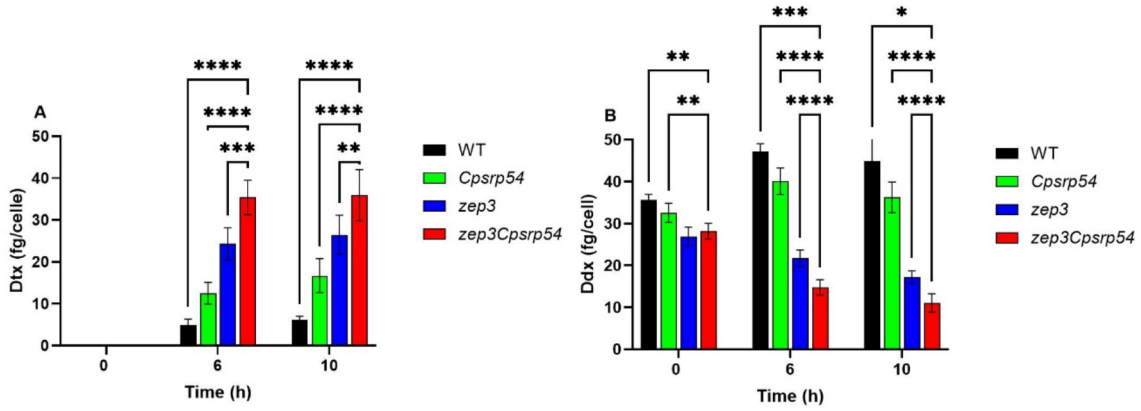


**Figure 1. Dtx and Ddx levels in WT, *Cpsrp54*, *zep3* and *zep3Cpsrp54* cells.** Dtx (A) and Ddx (B) concentrations (fg/cell) in WT, *Cpsrp54*, *zep3* and *zep3Cpsrp54* after exposure of LL acclimated cells (0 h) to 6 and 10 h of ML. Two independent mutant lines were included in the experiment for *Cpsrp54* and *zep3*, and four independent lines were included for *zep3Cpsrp54*. Three biological replicates were included for each of the independent mutant lines meaning that the total number of samples were n=6 for *zep3* and *Cpsrp54*; n=12 for *zep3Cpsrp54* and n=3 for WT.

*(Continued on next page)*

**Material and Methods**

*Zep3Cpsrp54* double KO mutants were generated by introducing mutations in the *CpSRP54* gene in the previously published *zep3* mutant lines using the CRISPR/Cas9 technology (Græsholt et al., 2024; Sharma et al., 2018). Bacterial conjugation was chosen as the method for delivery of the components necessary for CRISPR/Cas9-gene editing to take place to avoid incorporation of "foreign" DNA into the genome of the *P. tricornutum* cells (Sharma et al., 2018). The phenotype of the *zep3Cpsrp54* double KO mutant was characterized and compared to the phenotype of *zep3* and *Cpsrp54* single mutants and wild type (WT) cells. The phenotypic comparisons were performed by assessing their photophysiological performances using Pulse-amplitude modulated (PAM) fluorometry, pigment concentrations by HPLC and growth rates as a daily increase in *in vivo* chlorophyll *a* fluorescence as a response to different light treatments.

**Results and Discussion**

Non-transgenic *zep3Cpsrp54* double KO mutants with bi-allelic mutations were successfully generated using the CRISPR/Cas9 technology. The double KO mutants were found to display the photophysiological features of both *zep3* and *Cpsrp54* single mutants. The double KO mutants were unable to relax their Dtx-dependent photoprotective response in low light, a feature previously observed in the *zep3* single mutants (indicating that high light-accumulated Dtx was stabilized), and they were found to be light sensitive similar to the *Cpsrp54* single mutants (indicating that Dtx can be produced without the use of high intensity light) (Græsholt et al., 2024; Nymark et al., 2021). To test if the *zep3Cpsrp54* double mutants could function as a Dtx production line and outperform the single mutants and WT cells, we exposed low light (LL; 35 µmol photons $m^{-2}$ $s^{-1}$) acclimated mutants and WT to medium light (ML; 200 µmol photons $m^{-2}$ $s^{-1}$) for 6 and 10 h. Pigment analysis performed on material harvested from LL acclimated cultures and 6 h and 10 h ML treated cultures showed that ML was enough to trigger the double KO mutants to produce high levels of Dtx (Figure 1A). The highest concentrations of Dtx were found in the double KOs, followed, in decreasing order, by *zep3*, *Cpsrp54* and WT (Figure 1A). The opposite trend was observed for the Ddx concentration (Figure 1B). The Dtx production in the double KOs clearly outperformed the production in the *zep3* lines showing Dtx concentrations that were 46% and 36% higher than in *zep3* lines after 6 h and 10 h of ML treatment, respectively. The Dtx concentration in the double KO mutants after 6 h in ML reached similar levels as previously reported for *zep3* single mutants exposed to high intensity light (500 µmol photons $m^{-2}$ $s^{-1}$) (Græsholt, 2024). Even though the concentration of Dtx differed significantly between mutant lines, they were highly similar for the same mutant line at both 6 h and 10 h (Figure 1A). An additional experiment using shorter ML exposure times (0.5 h and 2 h) showed that prolonged exposure is necessary to reach maximum Dtx levels. Growth rates of the double KO mutants were at WT levels up to 150 µmol photons $m^{-2}$ $s^{-1}$ supporting the idea that these mutants could function as a production line of Dtx. Future project work will include large-scale cultivation of *zep3Cpsrp54* double KO mutants together with our industry partners to prove that commercial production of Dtx is a possibility.

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# EFFECTS OF FOUR TRADITIONAL CHINESE MEDICINES AND HARMFUL MICROALGAE *Karenia mikimotoi* ON THE GROWTH OF RAINBOW TROUT (*Oncorhynchus mykiss*) GILL EPITHELIAL CELL LINE, RTGILL-W1

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The fishery industry plays a crucial role in global food supply. According to the projections suggested by the Organisation for Economic Co-operation and Development (OECD), the marine aquaculture production will contribute 87% of global fish production by 2025. However, harmful algal blooms (HABs) formed by *Karenia mikimotoi* have caused substantial loss in aquaculture in various countries, including Japan, Norway, Ireland, New Zealand, and China. The economic impact has been severe, with losses exceeding USD 290 million in Chinese coastal waters in 2012 and USD 82.8 million in Hong Kong and surrounding areas in 1998. In 2015, the Agriculture, Fisheries and Conservation Department (AFCD) reported extensive cultured fish deaths caused by K. mikimotoi in nine fish culture zones across Tolo Harbour, Long Harbour, and northeastern Hong Kong waters.

*K. mikimotoi* is a bloom-forming dinoflagellate occurring worldwide that causes large economic losses through its lethal effects on marine fish, primarily through gill damage. In order to reduce losses and increase marine fish culture yields, algicides and antibiotics are often used in the aquaculture industry. However, these treatments may lead to great environmental impacts and development of antibiotic-resistant bacteria, affecting human health. Therefore, it is crucial to find an alternative to solve this problem. Traditional Chinese medicines (TCMs) may be the potential candidates. In this project, the protective effects of four TCMs, *Astragali Radix* (AR)*, Coptidis Rhizom*a (CR)*, Lonicera Japonica Flos* (LJF)*,* and *Poria* (P), were studied. The four TCMs were meshed into powders and soaked with *K. mikimotoi* (at 10,000 cells/mL) in the concentrations of 1 and 10 mg/mL for 6 and 24 hours. The screening results showed that AR, CR, and LJF have significant inhibitory effects on *Karenia mikimotoi* at 10 mg/mL.

The effects of the four TCMs on rainbow trout gill epithelial cell line, RTgill-W1 (CRL-2523) in the presence or absence of *K. Mikimotoi* was also explored. In the absence of HAB, the fish gill cells were treated with either 0, 0.1, 0.5, 1, 5, or 10 mg/mL of each selected TCM stocking solution for 24 hours. For algal challenge tests, 40,000 cells/mL of *K. mikimotoi* were added to the TCM-treated epithelial cells for a 2-hour incubation.

Three TCMs, including AR, P, and LJF, showed no cytotoxicity on RTgill-W1 at concentration of 1 mg/mL or below. Under algal challenging conditions, these three TCMs at 0.5 mg/mL demonstrated significant protective effects against *K. mikimotoi* compared to the untreated controls. Further research is needed to elucidate the underlying mechanisms.

# HARNESSING THE POTENTIAL OF SEAWEED IN OPEN WATER IMTA

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## Introduction

Integrated Multi-Trophic Aquaculture (IMTA) involves farming multiple aquaculture species from different trophic levels within the same production site or bay. In this system, uneaten feed, waste, and nutrients from one species (e.g., finfish) are utilised as resources—such as fertilizer or feed—for other species (e.g., seaweeds, suspension feeders, or deposit feeders). Feed and waste from the salmon create a nutrient flow across the Low Trophic Grid (LTG) in Lehanagh Pool. As an extractive species, seaweed absorbs dissolved inorganic nutrients, making it an ideal candidate for IMTA. Its ability to reduce nutrient loads from fed species contributes to environmental remediation and supports circular economy principles (Chopin & Tacon, 2020). The expansion of seaweed cultivation could act as a valuable and sustainable resource for the future of global aquaculture particularly in IMTA systems. This concept promotes balanced systems, enhances environmental sustainability, improving ecosystem services and contributes to a greater societal acceptance of aquaculture (Van Osch et al., 2019). This study investigated the growth of *Alaria esculenta* and *Saccharina latissima* in open water IMTA and assessed the nutritional and food safety quality of these species.

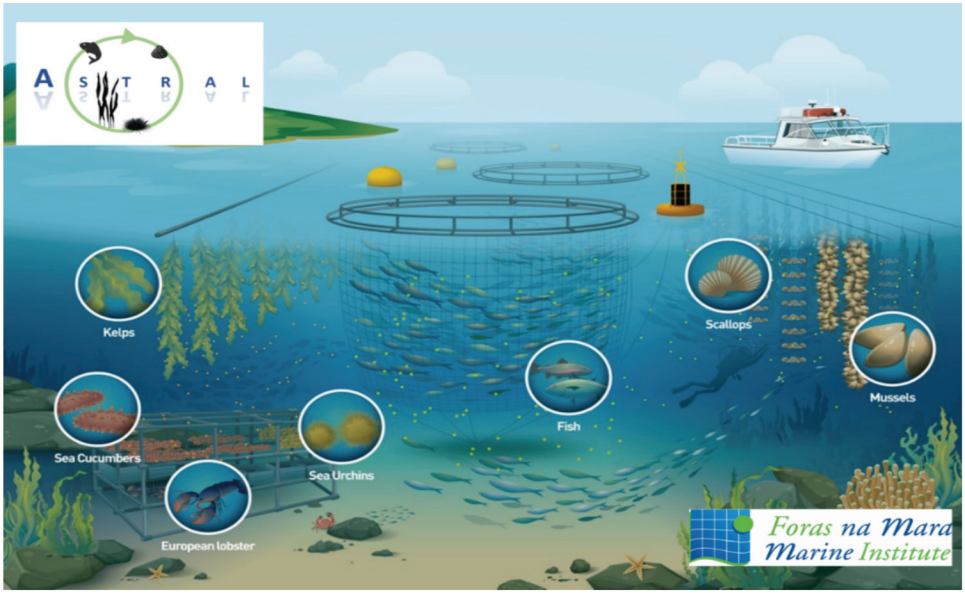


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**Table 1. Nutrient components of kelp dry weight (unless indicated) grown in the LTG and monoculture**

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# BIOACCUMULATION OF HEAVY METALS IN WATER COLUMN, SEDIMENT, AND TISSUE OF SILVER CATFISH (*Chrysichthys nigrodigitatus*) AND NILE TILAPIA (*Oreochromis niloticus*) COLLECTED FROM SAGBO KOJI RIVERINE COMMUNITY, LAGOS ISLAND, SOUTHWEST NIGERIA

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This study was conducted to assess the quantity of trace metals in the tissues of fish, water and sediments from Sagbo Koji Riverine Community, Lagos Island, Lagos State, Nigeria. Samples were collected from (8 weeks) in 2023. Eight samples each of *Chrysichthys nigrodigitatus* (Silver Catfish) and *Oreochromis niloticus* (Nile Tilapia) samples were purchased from local fishermen. Water and sediments samples were collected at two sampling points each. Heavy metal analysis was done on the fish tissues, sediments and water samples. Mean heavy metal concentration of water and sediment samples showed that zinc had the highest concentration of 1.636 mg/l and 18.135 mg/l while cobalt had the least of 0.009 mg/l and 0.552 mg/l respectively. Mean iron concentration of 114.152 mg/kg and 99.580 mg/kg was highest in Silver Catfish and Nile Tilapia respectively and the least was cadmium with 0.088 mg/kg and 0.015 mg/kg. Bio-concentration factor (BCF) of lead in fish against the water sample was 815.31 mg/kg for *C. nigrodigitatus* and iron with 685.82 mg/kg for *O. niloticus* was the highest. It was observed that the BCF of heavy metals found in fish sample compared to sediment had high values for lead (22.35 mg/kg and 16.39 mg/kg) for *C. nigrodigitatus* and *O. niloticus* respectively. The results from the study showed that only cadmium in fish samples, chromium in sediment samples, iron, copper, chromium, manganese and cobalt in water samples are within the WHO/FEPA maximum acceptable limit.

# DIETARY EFFECTS OF ORGANIC WASTE CULTURED BLACK SOLDIER FLY (*Hemertia illucensS*) (LINNAEUS, 1758) LARVAE MEAL ON SOME PHYSIOLOGICAL PARAMETERS OF HYBRID AFRICAN CATFISH *Heterobranchus bidorsalisS* ♂ (GEOFFROY SAINT-HILAIRE, 1809) × *Clarias gariepinus* ♀ (BURCHELL 1822)

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This study examined the effect of Black soldier fly larvae meal (BSFLM) on some physiological parameters in African catfish hybrid (*Heterobranchus bidorsalis* × *Clarias gariepinus*). Diets with BSFLM inclusion levels of 0, 20, 40, 60 and 80% denoted as BSFLM0 (Control), BSFLM20, BSFLM40, BSFLM60 and BSFLM80 were tested for 56 - days feeding. Fish fed BSFLM0 exhibited the highest growth and nutrient utilization among the diets, having a weight gain (WG) of 32.55 ± 0.29 g, feed intake (FI) of 54.73 ± 0.51 g, and specific growth rate (SGR) of 1.16 ± 0.35. No significant differences ($p>0.05$) was recorded between the fish fed BSFML0 and BSFML20 in their body weight, feed intake and histology. Higher BSFLM levels reduced performance, with BSFLM80 showing the lowest. Apparent digestibility coefficient (ADC) and histological examination revealed the same trend. These findings suggest that fishmeal can be replaced up to 20% with BSFLM, without compromising the growth, nutrient utilization and general health of hybrid African catfish.

# EDNA ON TRIAL: TOWARD A STANDARD PROTOCOL FOR BIOMONITORING IN SALMON FARMING

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## Introduction

Environmental DNA (eDNA) metabarcoding is emerging as a scalable, cost-effective tool for monitoring environmental impacts in salmon farming. While it offers advantages over traditional macrofaunal assessments, such as faster processing and broader biodiversity detection, uncertainty around protocol standardization, limits its operational use. Equally important is understanding how much variation naturally occurs between replicate sediment samples. To confidently implement eDNA in routine monitoring, we need to distinguish between biological variability and methodological noise, and ensure that protocol choices do not overshadow meaningful ecological signals.

## Materials and Methods

Sediment samples were collected from coastal salmon farms in western Scotland. Replicates were taken to assess within-site biological variability. Two different PCR protocols (primarily differing in DNA polymerase) were applied to the same DNA extracts to evaluate the effects of methodological variation on diversity metrics and community composition. Microbial communities were characterized via 16S rRNA gene sequencing. The results were compared against traditional macrofaunal assessments to evaluate eDNA's stability and suitability for environmental monitoring.

## Results

The two PCR protocols used in this study yielded statistically significant differences in bacterial community composition, indicating that protocol choice can influence eDNA-based assessments. Furthermore, the comparison of replicate samples showed that within-site variability in bacterial eDNA communities was comparable to that of macrofaunal assemblages collected according to compliance monitoring protocol (~25% variability in both cases), supporting the reliability of eDNA for detecting spatial patterns in benthic environments.

## Conclusion

Despite some sensitivity to laboratory protocols, eDNA-based biomonitoring offers reliable results with variability levels comparable to traditional methods. With protocol harmonization, eDNA has strong potential to serve as a scalable, cost-effective tool for routine environmental monitoring in the salmon aquaculture industry.

# ADVANCING AQUAFEED SAFETY: GENETIC DETECTION OF RUMINANT DNA FOR REGULATORY COMPLIANCE AND BIOSECURITY

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**Introduction**
The accurate detection of ruminant-derived ingredients in aquafeeds is critical for ensuring regulatory compliance, safeguarding biosecurity, and protecting brand integrity. The European Commission restricts the feeding of ruminant proteins to non-ruminant animals, making sensitive and reliable detection methods essential for producers operating in both domestic and export markets. While official methods, such as those developed by the Institute for Reference Materials and Measurements (IRMM), remain the regulatory benchmark, their primary focus is qualitative detection and morphological identification, which may limit their applicability for precise quantification in complex feed formulations.

**Materials and Methods**
We present a novel genetic diagnostic system based on real-time PCR that not only detects the presence of ruminant DNA with high specificity but also provides an optional quantitative estimate expressed as % weight/weight (w/w) of the product. This enables sensitive detection down to 0.1% w/w, allowing feed manufacturers to assess contamination levels and make informed decisions regarding product release, reformulation, or supplier management. Validation against a wide range of commercial aquafeeds confirms the robustness of the method across diverse matrices and ingredient profiles.

**Results**
By integrating a molecular approach alongside traditional IRMM methodologies, aquafeed producers can achieve faster turnaround times, increased sensitivity for trace-level contamination, and an enhanced capacity to demonstrate compliance. This innovation strengthens feed quality assurance programs, mitigates regulatory risk, and supports a more transparent and biosecure aquaculture supply chain.

**Conclusions**
Since discrepancies can exist between the results obtained from both approaches, it is essential to comprehensively evaluate the entire methodology, from sample extraction to final result interpretation, as well as the specific purpose of the analysis, to determine the most appropriate method for each application.

# PROTEIN QUALITY EVALUATION OF SUSTAINABLES MEALS FOR DIFFERENTS SPECIES OF MUGILIDAE

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**Introduction**

Currently, aquaculture faces two major challenges: the reduction of fish meal inclusion in feeds and the diversification of farmed species. Mugilids (family Mugilidae) emerge as a promising group to address these challenges, their omnivorous feeding habits allow a lower protein content in diets formulation. However, as has been observed in other species, the protein quality is critical to ensure proper growth, for this is essential to cover amino acid requirements. The objective of this study is to analyze the amino acid profiles of various sustainable feed ingredients and assess their suitability for three different mugilid species.

**Materials and methods**

The whole-body composition and amino acid profiles were analyzed for three fishes from three different wild mugilid species (*Mugil cephalus*, *Chelon labrosus*, and *Liza aurata*) at the laboratorie of the Universitat Politècnica de València (UPV). In parallel, the crude protein content and amino acid composition of different raw materials—vegetal, aquatic animal, terrestrial animal, and unicellular origin—were analyzed. Based on the amino acid data, the following nutritional indices were calculated: Amino Acid Ratio (AAR % = (AAsample)/(AAreference)*100), Chemical score (CS, %: Minimum value from AARs), Limiting amino acid (The amino acid that correspond to CS) and Oser´s Index (OI, % = $10^{(1/n*(\log(AAR1)+\log(AAR2)\ldots\log(AARn)))}$).

**Results**

The results obtained show that the most limiting amino acid in different raw materials was methionine (MET) followed by lysine (LYS) (Table 1), depending on the species. For *Mugil cephalus*, methionine was the most limiting amino acid while for *Chelon labrosus* were methionine and lysine and, finally, lysine was the limiting amino acid for *Liza aurata*.

In general, *Chelon labrosus* showed better IO and CS than the other two species for all raw materials. The IO index was higher in raw materials of aquatic animal origin (Figure 1) followed by Brewer's yeast 100%, Fermented soybeans, Potato protein and Beet pulp. For CS was similar than IO index and fish meal had the best CS. However, Brewer's yeast 100% showed better CS than krill and other raw materials had close to these values. and terrestrial raw materials and some vegetables raw materials had the worst values to OI index and CS.

**Discussion**

Results obtained indicate that, depending on the species, different dietary formulations are required to meet specific amino acid requirements. Normally, raw materials, which are not of marine origin, are deficient in methionine and lysine and those of vegetable origin have less protein. The protein and amino acid requirements vary according to the developmental stage of the fish and the specie. Therefore, it is necessary to utilize different combinations of raw materials even within the same species due to the substitution of fish meal with a single alternative protein source should not exceed 40% (Sánchez-Lozano et al., 2009; Valverde et al., 2013). One of the advantages of feed formulation in aquaculture is the ability to combine multiple raw ingredients to compensate for limiting amino acids and to ensure optimal growth performance.

*(Continued on next page)*

Table 1: Crude protein and limiting amino acid of *Mugil cephalus* (MC), *Chelon labrosus* (CL) and *Liza aurata* (LA)

| | CP(%d.m.) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| MC | 47.0 | MET | LYS | MET | MET | LYS | MET | MET | MET | MET |
| CL | 64.0 | ARG | LYS | MET | MET | LYS | MET | MET | MET | LYS |
| LA | 47.4 | ARG | LYS | MET | LYS | MET | LYS | ISO | LYS | HYS |
| | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| MC | MET | LYS | MET | MET | MET | MET | ISO | MET | LYS | MET |
| CL | LYS | LYS | LYS | HYS | ISO | MET | ISO | ARG | LYS | MET |
| LA | MET | MET | LYS | LYS | HYS | LYS | HYS | MET | MET | ISO |

Note: % d.m. dry matter; 1 Beet pulp; 2 Defatted cameline ; 3 Corn gluten meal; 4 Fermented soybean; 5 Hydrolyzed wheat protein; 6 Potato protein; 7 Soy protein concentrated; 8 Soybean meal; 9 Sunflower meal; 10 Wheat meal; 11 Wheat gluten; 12 Fish meal; 13 Krill meal; 14 Chicken meal; 15 Meat meal; 16 Porcine hemoglobin; 17 Brewer's yeast 100%; 18 Brewer's yeast 40%; 19 *Isochrysis galbana*.

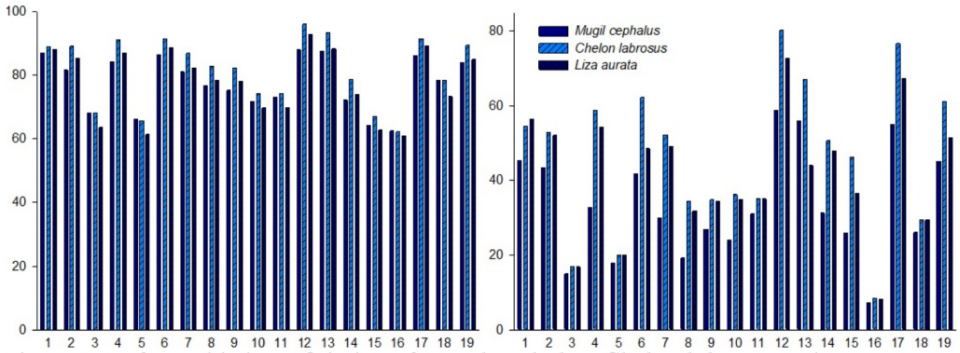


Figure 1: Left graphic bar, IO index of *Mugil cephalus*, *Chelon labrosus* and *Liza aurata*. Right graphic bar, Chemical score of *Mugil cephalus*, *Chelon labrosus* and *Liza aurata*.
Note: 1 Beet pulp; 2 Defatted cameline ; 3 Corn gluten meal; 4 Fermented soybean; 5 Hydrolyzed wheat protein; 6 Potato protein; 7 Soy protein concentrated; 8 Soybean meal; 9 Sunflower meal; 10 Wheat meal; 11 Wheat gluten; 12 Fish meal; 13 Krill meal; 14 Chicken meal; 15 Meat meal; 16 Porcine hemoglobin; 17 Brewer's yeast 100%; 18 Brewer's yeast 40%; 19 *Isochrysis galbana*.

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# STAGE-SPECIFIC RESPONSES OF SENEGALESE SOLE POSTLARVAE TO MICRO- AND MACROALGAE DIETARY BLENDS

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## Introduction

The Senegalese sole (*Solea senegalensis*) is a key species for aquaculture in Southern Europe. As production intensifies, there is an urgent need to develop tailored diets that support not only optimal growth but also resilience during common hatchery procedures such as transport, grading, or tank transfer. Previous studies have shown that certain additives such as curcumin, green tea extract, and grape seed extract can enhance growth and/or stress resilience in postlarval sole (Xavier et al., 2020; 2022). Micro- and macroalgae are rich in bioactive compounds and represent a promising dietary strategy to improve both growth performance and oxidative status in fish. Since the antioxidant system is essential for maintaining physiological homeostasis, especially under stressful conditions, designing diets adapted to specific production phases can improve hatchery protocols and overall biological efficiency. The aim of this study was to evaluate the effects of diets supplemented with a blend of micro- and macroalgae and to identify: (1) a growth-promoting diet suitable for the regular rearing phase, and (2) a functional diet that can enhance the resilience of postlarvae to routine aquaculture stressors.

## Material and methods

A feeding trial was conducted during the benthic phase of *Solea senegalensis* postlarval development (18-45 days after hatching, DAH) in a recirculating aquaculture system. Three experimental diets were tested in triplicate: a control diet (CTRL), a commercial-like formulation, and two diets supplemented with algae biomass, CTRL + *Ulva* sp.and *Tisochrysis lutea* (ULTISO), and CTRL + *Ulva* sp. and *Pavlova girans* (ULPAVL). Growth performance and antioxidant status were assessed at 38 DAH (Sampling 1-S1) to evaluate the effects of each diet during the rearing phase. From 38 to 43 DAH, all groups received a new diet enriched with a *Skeletonema costatum* extract (BOOST diet), aimed at preparing the fish for upcoming challenges. At 43 DAH, fish were subjected to a transport challenge (~24 h), followed by immersion in a virulent bacterial suspension (*Tenacibaculum maritimum*, strain ACC13.1) to induce tenacibaculosis at 44 DAH. A second sampling was performed at 45 DAH (Sampling 2-S2), 24 h post-infection, to evaluate the physiological responses to the BOOST diet under stressful and disease conditions. Growth performance at S1 was assessed through measurements of performance indicators and survival. Oxidative status was evaluated at both sampling points (S1 and S2) by quantifying the activity of superoxide dismutase (SOD) and catalase (CAT), along with levels of lipid peroxidation (LPO) as a marker of oxidative damage. Gene expression analyses were conducted at both timepoints for markers of antioxidant defense (*sod1*, *cat*, *gpx1*), epithelial integrity (*cldn15*, *ocl*, *tjp2*), and inflammation (*il1β*, *tnfα*).

## Results

At 38 DAH, postlarvae fed the ULPAVL diet showed significantly lower dry weight compared to the CTRL and ULTISO groups, which displayed similar values. However, dietary treatment had no significant effect on condition factor, relative growth rate, or survival. Redox status was influenced by diet: ULTISO-fed fish exhibited significantly higher activities of SOD and CAT compared to the ULPAVL group, although not significantly different from CTRL. Interestingly, LPO levels were significantly lower in the ULPAVL group compared to both CTRL and ULTISO fish. After feeding the BOOST diet and exposure to the transport and bacterial challenge, no significant differences were observed among treatments in SOD, CAT, or LPO levels at 45 DAH. At the molecular level, gene expression analysis at 38 DAH revealed no major differences among treatments, except for *cat*, which was significantly downregulated in both ULTISO- and ULPAVL-fed fish compared to CTRL. By 45 DAH, *cat* expression remained significantly lower in ULTISO-fed fish. In contrast, expression of *cldn15* was significantly higher in CTRL and ULPAVL groups compared to ULTISO.

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## Conclusion

While algae inclusion in diets did not enhance growth, survival, or overall antioxidant capacity in Senegalese sole postlarvae, both algae-supplemented diets modulated specific oxidative stress markers and gene expression. The ULTISO diet stimulated SOD and CAT activities at 38 DAH, indicating activation of the antioxidant system, but without reducing oxidative damage. Conversely, the ULPAVL diet lowered lipid peroxidation, suggesting a protective effect against oxidative stress. However, no diet emerged as a functional strategy to enhance resilience to common hatchery stressors. During the stress challenge phase, when all groups were fed the BOOST diet, no significant differences in antioxidant enzyme activities were detected among treatments. This indicates that the BOOST diet neither conferred measurable advantages over the control diet nor modulated carry-over effects of prior algae supplementation. These results highlight the challenge to obtain stage-specific diets, tailored to either promote growth or prepare fish for stressful production events, that will enhance robustness in marine hatcheries.

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# EFFECTS OF *Palmaria palmata* ON IMMUNITY AND GUT MICROBIOTA OF JUVENILE EUROPEAN SEABASS (*Dicentrarchus labrax*)

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## Introduction

The intensification of aquaculture has significantly contributed to meeting global fish demand. However, it has also increased disease outbreaks, particularly in economically important species such as European seabass (*Dicentrarchus labrax*) [1]. Conventional disease management practices, such as the use of antibiotics and chemotherapeutic agents, have raised serious concerns due to the emergence of antibiotic-resistant pathogens and environmental contamination [2]. In response, there is growing interest in sustainable alternatives that promote fish health and reduce reliance on synthetic treatments [3]. Among these, dietary supplementation with natural bioactive compounds has shown potential to enhance antioxidant status, modulate immune function, and improve growth performance [4]. Marine macroalgae are a promising source of bioactive compounds such as polysaccharides, pigments, vitamins, and minerals, which exhibit immunostimulant, antimicrobial, and antioxidant activities [5].

Beyond their general nutritional value, certain macroalgae possess immunomodulatory properties, primarily due to their content of phenolic compounds and complex undigestible polysaccharides such as laminarin, fucoidan, and alginates, which have an immune-modulatory effect in fish [6]. Even at low dietary inclusion levels, macroalgal supplementation has been linked to improved immune function and increased resistance to pathogens [7]. However, the high content of indigestible structural polysaccharides in macroalgal cell walls may limit the accessibility of these bioactive compounds. Therefore, pre-treatment methods such as enzymatic hydrolysis, fermentation, or alkaline treatment are employed to improve the release and bioavailability of immune-stimulating components. [8]. Therefore, the present study aimed to evaluate the effects of dietary inclusion of *Palmaria palmata*, pre-treated using an alkaline hydrothermal pre-treatment followed by autoclaving, on the juvenile European seabass's intestinal immune status and microbiota composition.

## Materials and Methods

*P. palmata* underwent alkaline hydrothermal pre-treatment (1N NaOH; autoclaving at 121 °C for 30 minutes) and was dried before its incorporation in the diets. Five experimental diets were formulated to be isoproteic (48% on a dry matter basis) and isolipidic (18% on a dry matter basis), including a control diet with no *P. palmata*, and four test diets containing 7.5% or 15% of either untreated or pre-treated *P. palmata*, replacing a plant ingredient mixture of the control diet. European seabass (*Dicentrarchus labrax*) with an initial average weight of 38 g were randomly assigned to triplicate groups of 16 fish per tank. The feeding trial lasted for 11 weeks, during which fish were hand-fed to apparent visual satiation, twice daily, six days per week. At the end of the trial, blood/plasma and intestines from three fish per tank were collected and stored at −80 °C for subsequent immune and microbiota analyses. A small portion of distal intestinal tissue from the same fish was stored in RNAlater for the assessment of *TNF-α*, *IL-8*, *IL-1β*, *IL-10*, *COX-2*, *casp9*, and *casp3* gene expression by reverse transcription quantitative PCR (RT-qPCR). Primer efficiency and reference gene stability were verified to ensure accurate data normalization. Innate humoral immune responses, including antiprotease, protease, peroxidase, and lysozyme activities, were quantified using established spectrophotometric methods. Microbial community profiling was performed via Denaturing Gradient Gel Electrophoresis (DGGE) of 16S rRNA gene fragments amplified from bacterial DNA extracted from intestinal digesta and mucosa.

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Data were tested for normality and homogeneity of variances and normalized when required. Treatment effects were assessed using two-way ANOVA, considering *P. palmata* inclusion level and pre-treatment as fixed factors, followed by Tukey's test when $p < 0.05$. Non-orthogonal contrasts were performed to compare the control with the test diets. Statistical analyses were performed using IBM SPSS Statistics v26. Microbiota analysis used DGGE banding patterns [9]. Band intensities were analyzed with Quantity One software and converted into presence/absence matrices. Primer v7 was used to calculate group similarities and non-metric multidimensional scaling (MDS) based on Bray-Curtis similarities. Diversity indices included Shannon-Weaver and Margalef, while similarity percentages were assessed using SIMPER. DGGE clustering was done via Bray-Curtis dendrograms using UPGMA.

**Results**

The dietary *P. palmata* levels or pre-treatment did not affect plasma immune parameters, including peroxidase, antiprotease activity, protease activity, and lysozyme. However, fish fed non-treated *P. palmata* exhibited higher intestinal expression of *IL-10* and *casp3* than those fed the corresponding pre-treated diets. The intestinal expression of *TNF-*□, *IL-8*, *IL-1*□, *COX2*, and *casp9* was not significantly affected by diets.

In the intestinal digesta microbial community analysis, fish fed the non-treated 15% *P. palmata* diet had higher similarity among replicates than those fed the control diet. No significant differences in similarity were observed between the control and the non-treated 7.5% *P. palmata* or pre-treated 7.5% and 15% *P. palmata* groups. Dietary treatments did not affect the number of OTUs, richness, and diversity in the intestinal digesta. In the intestinal mucosa, richness and similarity were lower in fish fed the pre-treated 15% *P. palmata* than in those fed the control diet. However, no significant differences were found when comparing the control diet to non-treated 7.5% *P. palmata* and treated 7.5% and 15% *P. palmata* diets. Dietary treatments did not affect the number of OTUs and microbial diversity in the intestinal mucosa.

**Conclusion**

Overall, non-treated *P. palmata* induced a more consistent immune stimulation than the pre-treated *P. palmata.* Further studies are required to clarify the potential benefits of using treated or untreated *P. palmata* in European seabass juvenies diets.

**Acknowledgment**

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# MODULATION OF SEABASS IMMUNITY WITH PRE-TREATED *Codium tomentosum*

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## Introduction

Including macroalgae in aquaculture diets has shown potential to enhance the immune system of farmed fish. Rich in bioactive compounds such as pigments and other antioxidant compounds, macroalgae can improve fish immune responses by enhancing antioxidant defenses and increasing resistance to pathogens [1]. Studies have shown that dietary supplementation with macroalgae extracts enhances disease resistance, survival, and stress resistance [2]. These natural immunomodulatory effects are particularly relevant given the frequent stressors and disease outbreaks in farming conditions that can compromise fish immune function, as well as the increased awareness regarding the issues of synthetic additives for aquafeeds [3].

However, the bioavailability of macroalgae functional compounds is limited due to the complex polysaccharide matrix of macroalgae cell walls, which is not digested by fish [4]. To improve the availability of nutrients and biocompounds, treatments that break down algal cell walls are necessary [5]. These include mechanical, thermal, chemical, and biological methods [6,7]. Among them, alkaline chemical treatments are particularly effective, as they partially degrade structural carbohydrates such as hemicellulose and cellulose. This structural modification can improve cell wall permeability, thereby facilitating the release of antioxidants and enhancing nutrient availability [4,8]. The present study aimed to investigate the effects of dietary inclusion of *Codium tomentosum* pre-treated with alkaline solution and autoclaved on immune status and microbiota composition in European seabass (*Dicentrarchus labrax*) juveniles.

## Materials and Methods

*Codium tomentosum* was subjected to an alkaline (1N NaOH) hydrothermal pre-treatment and autoclaved at 121ºC for 30 or 60 minutes. Four isoproteic (48% dry matter basis) and isolipidic (18% dry matter basis) diets, namely, a control diet, with no inclusion of *C. tomentosum,* and three other diets including 7.5% untreated or pre-treated *C. tomentosum* replacing a plant feedstuff mixture of the control diet were used and fed to triplicate groups of 16 European seabass (initial body weight of 38g). The trial lasted 11 weeks, and during the trial, fish were hand-fed twice daily, 6 days a week, until apparent visual satiation. At the end of the growth trial, blood from 3 fish per tank was collected from the caudal vein using heparinized syringes, centrifuged at 6800 x g for 10 min, and the resulting plasma stored at -80ºC until analysis. The intestines of the same fish were also collected and stored in RNAlater for gene expression analysis. The expression levels of the following genes were evaluated by rt-PCR: *TNF-α, IL-8, IL-1β, IL-10, COX-2, casp3, and casp9*. Primer efficiency and reference gene stability were validated to ensure reliable normalization. Plasma innate immune parameters (antiprotease, protease, peroxidase, and lysozyme) were measured using standard spectrophotometric assays.

A log transformation was applied when necessary after testing for normality and homogeneity of variances. Data were submitted to a one-way ANOVA, followed by Tukey's test when $p < 0.05$. Analyses were conducted using IBM SPSS Statistics v26.

## Results

Fish fed the two pre-treated *C. tomentosum* diets showed significantly higher pasma peroxidase activities, both of which were greater than those of the non-treated group. Antiprotease activity was lowest in fish fed the diet including pre-treated *C. tomentosum* autoclaved for 30 minutes, while the highest value was observed in the non-treated *C. tomentosum* diet. Protease and lysozyme activity were not significantly affected by diet composition.

*(Continued on next page)*

The intestinal expression of *IL-8* was upregulated in seabass fed the *C. tomentosum* pre-treated and autoclaved for 30 minutes diet compared to the other three diets. On the other hand, intestinal expression of *IL-1β* and *casp3* was higher in seabass fed the non-treated *C. tomentosum* diet than in those fed the control and *C. tomentosum* pre-treated and autoclaved for 30 minutes diets, respectively. No significant differences between dietary treatments were observed in the intestinal expression of TNF-α, IL-10, COX-2, or casp9.

**Conclusion**

The results suggest that pre-treating *C. tomentosum* with an alkaline solution, followed by 30 minutes of autoclaving, produces a complex yet potentially advantageous immunomodulatory effect. Overall, *C. tomentosum* appears to enhance plasma peroxidase activity and help regulate inflammatory responses, supporting its promise as a functional dietary ingredient for aquafeeds.

**Acknowledgment**

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# OPERATIONALIZING CLIMATE CHANGE ADAPTATION AND MITIGATION FOR AQUACULTURE – PRACTICAL SOLUTIONS

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The Operationalizing Climate Change Adaptation and Mitigation for aquaculture (OCCAM) Innovation Action is funded by the Horizon Europe funding programme and consists of 22 industry and research partners from across Europe. The OCCAM objective is to implement, test, and evaluate practical and innovative solutions for climate change adaptation and mitigation in the aquaculture sector, and to deliver scalable, replicable, and exploitable solutions with accompanying recommendations ready for deployment in a wide variety of aquaculture supply chains. Some solutions are generic in nature with application across countries and sectors; this includes a Good Practice recommendation for self-assessment of CO2 emissions, guidelines for how to meet sustainability reporting requirements, templates for requesting and providing scientific advice, and a policy recommendation focusing on gaps and opportunities for climate-resilient aquaculture production. Other solutions are specific for sectors, species, and production methods, including solutions for carp, rainbow trout, salmon, bivalves, and seaweed (Ulva). To reduce emissions and contribute to aquaculture sector mitigation of climate change, sediments from freshwater aquaculture are upcycled, sludge is used to produce biochar, a digital tool for self-assessment of emissions from bivalve production is developed, and nets and pens with significantly reduced emissions are produced. Adaptation solutions include assessment of water availability and quality for freshwater aquaculture, selective breeding of more resilient rainbow trout, digital tools for forecasting and managing sea lice and Harmful Algal Blooms,and change of deployment parameters for oysters and seaweed given the expected changes in temperature and other environmental conditions. A prototype or proof of concept is developed for each solution, and where possible the proposed solution is also evaluated at a different site, and general recommendations are provided to other industry actors wishing to implement similar solutions.

OCCAM started in 2025 and will end in 2029, and in addition to the 22 project partners an Industry Reference Group (IRG) is being established, consisting of aquaculture industry partners interested in testing and evaluating mature prototypes of the various solutions. The IRG will be invited to stakeholder workshops and will be invited to give feedback on the functionality and the potential application of the respective solutions and will thus have an opportunity to influence the final version of these. Membership in the OCCAM IRG is free and might be of particular interest for producers of carp, rainbow trout, salmon, bivalves, and macroalgae.

# PRELIMINARY STUDY ON THE EFFECT OF RAW CHITIN FROM *Hermetia illucens* EXUVIAE AND LAURIC ACID ON GUT MORPHOLOGY AND FUNCTIONALITY IN RAINBOW TROUT (*Oncorhynchus mykiss*) FED SEMI-PURIFIED DIETS

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## Introduction

Natural functional feed additives can enhance both fish growth and health (Onomu et al., 2024) through the interplay between bioactive compounds and intestinal mucosa. Insects and their derivatives are considered a promising feed ingredient and additive to promote animal growth and health enhancement (Veldkamp et al., 2021).This study aimed to describe the effects of two compounds derived from black soldier fly (*Hermetia illucens,* BSF): a polysaccharide (chitin from exuviae, CHI) and a fatty acid (lauric acid, LAU). A feeding trial was performed with juvenile rainbow trout (*Oncorhynchus mykiss*) to assess if and to what extent raw chitin from BSF used alone or in combination with increasing levels of lauric acid can affect liver and intestinal morphology by focusing on goblet cells mucus composition, intestinal cell proliferation and differentiation as biomarkers.

## Materials and methods

Four semi-purified diets were formulated for juvenile rainbow trout to be grossly isoproteic (44%) and isolipidic (14%). Starting from a control basal diet (CTRL), three other test diets were produced to include a fixed amount of raw chitin from exuviae of BSF (1.5% dietary inclusion) (C-CHI) without lauric acid or with increasing level of it at 0.1% and 0.2% (C-CHI-LAU1 and C-CHI-LAU2; respectively). A total of 144 rainbow trout juveniles (91.3 ± 2.9 g) were randomly distributed into 12 fiberglass tanks (0.2 $m^3$; 3 tanks per dietary treatment) and fed over 10 weeks at a daily feeding rate of 2% body weight. At the end of the trial, growth parameters were measured and intestinal samples from pyloric caeca (PC) and proximal intestine (PI) were collected and immediately fixed in formalin for histological and immuno-histochemical analyses to assess the gut mucosae status. In particular, hematoxylin-eosin-stained sections were used to evaluate the general morphological aspects, PAS combined with Alcian Blue pH 2.5 staining to analyze the overall complex carbohydrates, and immunostaining for proliferating cell nuclear antigen (PCNA) and peptide transporter 1 (PepT1) to assess cell proliferation and peptides absorption respectively. Finally, the histochemical detection of alkaline phosphatase (ALP) was used to detect fully differentiated enterocytes.

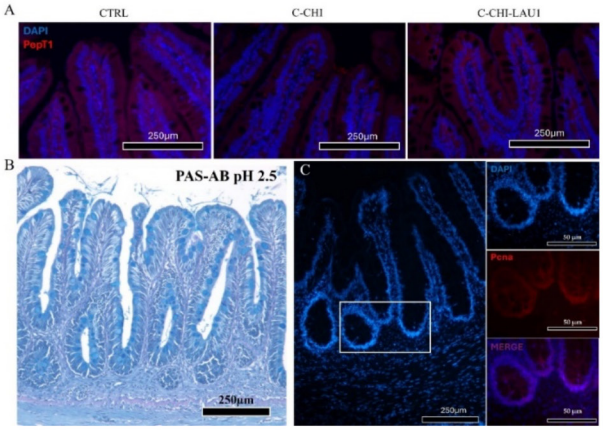


Figure 1: A) immunolocalization of PepT1 in the different dietary treatments, B) PAS combined AB pH 2.5 staining showing a heterogeneous mucus composition, and C) immunolocalization of PCNA restricted at the base of the folds.

*(Continued on next page)*

**Results**

There were no significant differences in the growth performance and body indices between the fish fed the control and the experimental diets. There were no histomorphological changes in the liver of the fish fed the different diets. However, there was a significant reduction in villi length ($p<0.05$) and changes in the mucus composition of goblet cells in the PC and PI ($p<0.05$). Lauric acid (0.2%) in the diet increased the number of mixed mucins in the PC, while chitin (1.5%) significantly increased the acid mucins alongside a significant decrease in mixed mucins in the PI ($p<0.05$). The signal intensity of peptide transporter 1 (PepT1) was lower in fish fed with the C-CHI diet (Fig 1). The PCNA and ALP expressions in PI did not differ significantly among dietary treatments ($p>0.05$).

**Discussion and Conclusion**

This study showed that including BSF exuviae and LAU into trout diets resulted in no differences in growth performances and biometric indices, similarly to what was observed in the same species by Rimoldi et al. (2023) with an inclusion of 1.6%, but unlike what was reported in sea bream fed diet including 0.02% LAU (Ullah et al., 2025).

Raw chitin alone caused a reduction in the folds length, and it affected goblet cell mucin production in rainbow trout fed diet with BSF meal (Elia et al., 2018). Furthermore, raw chitin decreased PepT1 signal intensity, indicating reduced intestinal peptide absorption, similar to what was observed when trout were fed a plant-protein based diet (Verdile et al. (2023). None of the diets affected PCNA and ALP expression, indicating that both raw chitin or LAU, did not induce proliferative state of intestinal tissue and cellular damage in the rainbow trout intestine (Kilemade et al. 2002).

Overall, these findings indicate that 1.5% BSF exuviae supplementation can negatively impact intestinal morphology, mucin production, and PepT1 expression, whereas a combination with 0.1% LAU in the diet seems to ameliorate the adverse effects of chitin.

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# SOUND AS A POSSIBLE STRESS AND WELFARE CHALLENGE IN SALMON FARMING

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**Noise in salmon farming**
In modern aquaculture, both at sea and on land, farmed salmon live in environments characterized by significant human-made noise. This noise may potentially have a negative impact on the most farmed animal in Norway. However, the extent and effects is not fully understood. Our aim was to describe the typical sounds within salmon aquaculture, develop methods to investigate long-term effects of noise, test habituation to noise and summarize present knowledge within the field.

**Methods and setup**
Firstly, sound was measured over representative periods at 10 different farms divided among open or closed sea cages and land-based systems. Secondly, we exposed salmon experimentally to known stressful low-frequency sound (10 Hz) for 5 minutes daily, to better understand the responses. Behaviour and physiology, together with molecular markers in the brain were to be identified for stress assessment. Thirdly, salmon smolts were experimentally exposed to "fish-farming sounds" in a predictable or unpredictable setup in freshwater tanks, followed by unpredictable sounds in sea cages to study both short- and long-term effects on stress and habituation. Finally, a review was written.

**Results and discussion**
The sound farmed salmon experience ranges from background levels around 95 dB re 1 µPa to typical levels approaching 134 dB, with very noisy periods up to 157 dB. These levels range within levels measured in other studies but provide further details. Closed sea cages were noisiest, likely caused by pumps continuously supplying water. Land-based systems were somewhat less noisy and open cages quietest, especially at night, though noisy operations and boats were present during the day. On three occasions, extremely high sounds exceeding 175 dB were measured, either when an underwater door was slammed shut or when blastings occurred nearby. The frequency range of the noise fell within the audible range for salmon. Overall, farmed salmon live in a noisy world, with occasionally very high sounds.

Low frequencies (<15 Hz) are particularly stressful for salmonids, and salmon exposed to 142-169 dB re 1 µPa immediately responded with flight reactions, elevated plasma cortisol levels, and reduced neuronal activity in the hypothalamus. Over time, fewer fish reacted to the daily repetitive, familiar sound, and the cortisol physiological stress response diminished. Salmon thus showed behavioural and physiological habituation. However, after a month and 30 exposures, the salmon still had increased serotonergic activity (indicating chronic stress in the brain) and suppressed expression of genes in the hypothalamus that are normally positively associated with growth and reproduction.

Freshwater parr-smolts were experimentally exposed to predictable, unpredictable or control tank sounds, followed by exposure to unpredictable sounds in sea cages. No group differences in the freshwater phase were present, but similar behavioural reactions and no physiological responses in cortisol or neurochemical signalling of serotonin or dopamine were seen. When individuals were further acutely stressed, normal physiological responses suggested that none of the sound environments were more stressful during the freshwater phase. In sea cages the most interesting (and unexpected) finding was that control fish reacted behaviourally the least, while fish that experienced sound in freshwater responded relatively strongly with a disorganized swimming pattern, more so in predictable compared to the unpredictable group. Cortisol responses in seawater were absent when exposed to boat noise or similar, but normal when additional acute stress was given. The neurochemical signalling responses showed that after the acute stress test, both the control and unpredictable groups responded with increased serotonergic activity in specific areas of the brain, while fish exposed to predictable sound did not. Elevated cortisol levels without a corresponding increase in serotonin levels may be harmful by impairing brain health (reduced neuronal plasticity and imbalance in the body's energy budget). Behavioural and neurochemical responses suggest that fish exposed to high levels of predictable sound during the freshwater phase become more stressed when exposed to unpredictable sound in the seawater phase.

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The review reveals that most research has been measured in sound pressure (dB re 1 μPa), while particle movement ($ms^{-2}$), the most important sound component to which salmon respond, is scarcely studied. Most studies are on physoclistous fish, having hearing thresholds far below the physostomous salmon. Salmonids can detect frequencies from 1 to 1000 Hz, but with lowest thresholds between 100 to 500 Hz, frequencies that overlap noise in aquaculture. Cumulative exposures to sound levels exceeding 177 re 1 $\mu Pa^2s$ caused moderate physiological damage, while levels exceeding 183 re 1 $\mu Pa^2s$ caused lethal damage in chinook salmon. Acute sounds typically trigger a behavioural flight or stress response, where salmon try to avoid the noisy area or swim in disrupted directions and speed. Long-term studies exposing salmon to ≤149 re 1 μPa triggered behaviour that disappeared over time, while physiology and welfare were unchanged (except for the study mentioned above with 142-169 dB re 1 μPa). A few studies report that infrasound (e.g., 5 Hz) is particularly frightening for salmon, supporting the long-term study where daily exposure to 10 Hz resulted in poorer welfare. Habituation to sound is common, both in terms of behaviour and physiology.

The combined works compile a list of recommendations for noise in salmon aquaculture. Noise measurements within the audible range for salmon should be considered against "established" threshold values for known negative consequences on behaviour, physiology, and physical damage. Unnecessary high noise should be avoided, and unpredictable noise minimized. Fish farmers should actively manage the use of noise sources near the fish and consider whether habituation is possible. Blasting work nearby or other acute high sounds can lead to undesirable behavioural changes or injuries and should be avoided. Low noise levels in freshwater may result in more resilient salmon in seawater. Mitigations could include identifying noise sources with frequencies < 500 Hz and dampening the sound by separation or physical attenuation. A good example of noise reduction is to soften the closing of hatches and avoid them slamming shut with a bang.

We must better understand the long-term consequences of different sound environments in the freshwater phase (high vs. low noise). We have already observed that very loud sounds affect the brain in a way that may hinder growth or impair brain health, though the long-term consequences have not been studied.

Normal sounds in aquaculture (<135 dB re 1 μPa) are not expected to have significant negative consequences for the salmon, but short noisy periods (<157 dB re 1 μPa) could have negative effects depending on their duration, while the acute sounds observed (>175 dB re 1 μPa) could lead to hearing loss and physical damage. The knowledge compilation highlights research needs for more precise threshold values of how frequencies and durations affect salmon behaviour, physiology and welfare. Advisory knowledge for blasting is insufficient. Standardized measurement methods should be developed to enable fish farmers to measure relevant noise that could affect salmon, choose mitigation measures, and assess their effects. Data on particle acceleration and its consequences, the most important sound component for salmon, is lacking.

# PHOTOBACTERIOSIS DYNAMICS IN EUROPEAN SEABASS (*Dicentrarchus labrax*) IN CROATIA IN LAST FIVE YEARS

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**Introduction**

Photobacteriosis is a globally distributed infectious disease caused by the Gram-negative halophilic bacterium *Photobacterium damselae* subsp. *piscicida*. In Mediterranean marine aquaculture, it poses a serious threat, especially when sea temperatures rise above 22°C (Romalde, 2002). In the Adriatic Sea, the disease primarily affects seabream fry in hatcheries and seabass during the ongrowing phase.

Outbreaks are often triggered by prolonged high temperatures, low salinity, and poor farming conditions such as low oxygen levels and handling stress. Infected fish show signs of hyperpigmentation, lethargy, and swimming imbalance. As the disease advances, symptoms include anorexia, darkened skin, skin erosions, pale gills with excess mucus, and necrotic patches. Dead fish are typically found at the bottom of cages. Necropsy findings in acute cases include mild hemorrhages and splenomegaly with small white necrotic foci. Chronic cases show enlarged spleens with coalescing nodular white lesions (Galeotti et al., 2022). Acute mortalities often go undetected, and without prompt treatment, fry losses can reach 70–80%.

The Interreg project MARINET aims to develop an AI-based risk assessment model using environmental data from multiparametric sensors and daily monitoring of operational welfare indicators (OWIs) as defined by Manfrin (2022). This study seeks to establish a baseline for the model using five years of data from Croatian Adriatic farms.

*Material and methods*

All samples submitted to the Croatian Veterinary Institute for diagnostic testing that tested positive for photobacteriosis between 2020 and 2025 were included in this study. The samples originated from marine fish farms located along the Croatian coastline of the Adriatic Sea.

Each sample underwent a standardized diagnostic protocol (Varvarigos 2020). Anamnestic data were collected, followed by weighing of the specimens and documentation of any external abnormalities. Subsequently, necropsies were performed. Observations made during necropsy were recorded, and a presumptive diagnosis was confirmed through bacteriological examination. This involved isolating bacteria on Marine Agar and Tryptic Soy Agar (TSA) supplemented with 1.5% NaCl. Preliminary identification was conducted using the API 20E system, along with an assessment of the bacteria's ability to grow on selective media such as Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar. Final confirmation of *Photobacterium damselae* subsp. *piscicida* was achieved through PCR analysis, following protocols described by Andreoni and Magnani (2014). Co-infections were also confirmed.

All collected data were analyzed using descriptive statistical methods.

*Results*

Between 2020 and 2025, a total of 51 photobacteriosis outbreaks in seabass were diagnosed in our laboratory. Of these, 25 occurred in 2024, six in 2023, none in 2022, four in 2021, and six in 2020. Most of the affected fish weighed less than 100 grams, with only two cases involving larger specimens. Outbreaks most frequently occurred in late summer and autumn, when sea temperatures ranged from 18°C to 26°C. Chronic cases were typically observed in cooler periods (autumn to spring), while acute cases were more common at higher temperatures (up to 26°C) (Fig. 1). Co-infections with *Vibrio harveyi* and/or *Tenacibaculum maritimum* were significantly more common in 2024 compared to previous years, likely due to the prolonged period of elevated sea temperatures during summer and autumn (Fig. 2).

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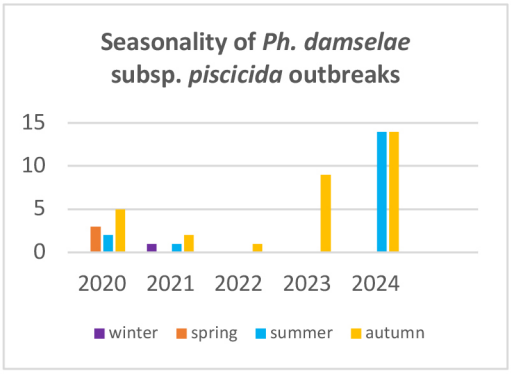


**Fig 1. Seasonality of photobacteriosis during last five years**

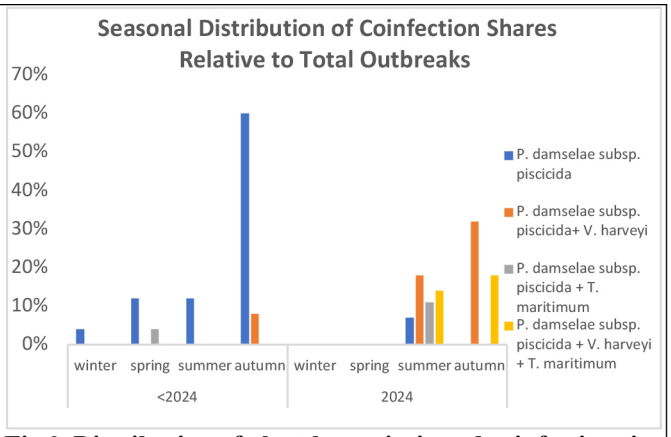


**Fig 2. Distribution of photobacteriosis and coinfections in 2024 and period from 2020-2023**

The disease was characterised by external lesions ranged from petechial hemorrhages on the head, operculum, and pectoral fin bases to more extensive hemorrhaging along the flanks, ventral fins, and around the anus. Gills appeared pale, occasionally with hemorrhagic patches. Necropsy findings varied from splenomegaly with barely visible whitish nodules to hemorrhages in the liver and intestines. Overall, the data suggest that the early onset of elevated sea temperatures acts as a trigger for disease outbreaks, exacerbated by co-infections with opportunistic pathogens such as *V. harveyi* and *T. maritimum*. In some cases, triple bacterial infections were observed, leading to severe losses.

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# A NOVEL IN-SITU OXYGENATION SYSTEM FOR RAS AND TANK AQUACULTURE

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Oxygenation in tank culture systems (e.g. RAS and race-ways) is one of the key factors affecting fish growth and welfare, and one of the major important production costs. Recent developments in RAS tank design faced the problem about how to make dissolved oxygen available for the animals in the tank (in-situ), especially in shallow (1.5m) and large surface area, or deep (4-6m) and circular tanks.

The analysis of available oxygenation solutions and potential new oxygen transfer technologies considered relevant aspects such as oxygen input efficiency and dissolved gas distribution over time and space in the tank, as well as energy demand, individuality, and independency.

After a thoroughly analysis, a novel in-situ oxygenation system was developed. It consists of an independent low power consumption unit with a ceramic membrane that operates at both low gas and water pressure. Table I shows the amount of oxygen input level and energy demand of the oxygenation system (named Canox).

As a self-sufficient and plug-and-play device, the unit can be quickly installed and retrofitted into existing tanks. The Canox oxygenation device is installed directly in/at the tank and uses the internal tank circulation and water flow for a very even oxygen distribution, ensuring a good oxygen supply. Due to the low gas and water pressure, there are no generation of harmful pressure release bubbles. Moreover, the normal behaviour of the fish and shrimp were not affected. The unit works completely independent of the main water circulation system and transfers oxygen into the tank even if the main water flow is stopped.

Existing technologies (low head oxygenators, U-tubes, oxygen cones, among others) were found to be inappropriate for an in-situ oxygenation. Other technologies were characterised by an extremely high cost at similar gas input efficiency levels.

Table I. Oxygen input level (L/h, kg/h of oxygen), energy demand (kg of oxygen/kW), and efficiency (%) for two different Canox models.

| Model name | Oxygen input | | Energy demand | Efficiency |
|---|---|---|---|---|
| | L/h | kg/h | kg/kW | % |
| Canox 20[1] | 300-600 | 0.4-0.8 | 8 | 70 – 100[2] |
| Canox 40 | 4.500 | 6.0 | 3.4 | 70 – 100[2] |

[1] with motor controller, adjustable between 5-100% of duty
[2] values will depend on tank size, load, and unit configuration

# AQUEOUS ALMOND (*Terminalia catappa*) LEAF EXTRACT ENHANCED GROWTH PERFORMANCE, IMMUNOCOMPETENCE AND RESISTANCE OF *Heterobranchus longifilis* TO *Pseudomonas aeruginosa*

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**Introduction**

The growing demand for sustainable aquaculture has led to increased interest in natural feed additives. *Terminalia catappa* is a promising plant with bioactive compounds like flavonoids and tannins that can enhance fish health and growth Abdel-Tawwab *et al*, 2018; Omitoyin *et al*., 2019; Ajani *et al*., 2020). However, extraction methods significantly influence the quality of the extract (Bae *et al.,* 2012). This study aims to evaluate the impact of different *T. catappa* extracts on the growth, health, and disease resistance of *Heterobranchus longifilis*. By investigating the effects of these extracts on growth performance, gut morphology, hematology, and resistance to *Pseudomonas aeruginosa*, this research contributes to the development of more sustainable and efficient aquaculture practices.

**Materials and methods**

Five experimental diets were formulated with varying concentrations of ethanol and aqueous extracts of *Terminalia catappa*. Diets were fed to triplicate groups of *Heterobranchus longifilis* for 70 days. Growth parameters, feed utilization, and hematological indices were measured. Liver samples were analyzed for antioxidant enzyme activity (SOD, GST, GPx) and lipid peroxidation (MDA). Fish were challenged with *Pseudomonas aeruginosa* to assess their disease resistance. Data were analyzed using one-way ANOVA and Duncan's Multiple Range Test to determine significant differences among treatments.

**Results and Discussion**

The study compared the efficacy of ethanol and aqueous extracts of *Terminalia catappa* on *Heterobranchus longifilis*. Ethanol extracts were found to have higher concentrations of bioactive compounds, but negatively impacted feed intake and growth performance. In contrast, aqueous extracts improved growth, feed utilization, and survival rates. This can be attributed to the varying polarity in the solvents. According to Archundia *et al,* (2019), the polarity in ethanol be described as medium while it is high in water. This suggests that aqueous extracts of *T. catappa* may be a more suitable dietary supplement for enhancing fish growth and health.

Poor nutrient utilization in ethanol extract-based diets led to reduced growth due to high levels of bioactive compounds like phenols. According to Thakur et al. (2019), these compounds act as anti-nutrients at high concentrations. Antioxidant indicators like GST and SOD were higher in aqueous extract diets, showing oxidative stress protection, which is required in fish during the normal feeding cycle. Fish fed T. catappa extracts showed increased resistance to *P. aeruginosa*, improving survival rates.
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Table 1: Result of the quantitative screening of ethanoic and aqueous extracts of *T. catappa*

| **Phytochemical** | Ethanoic extract concentration | Aqueous extract concentration |
|---|---|---|
| Total Phenolics (mg/g GAE) | 89.139±0.01 | 42.462±0.00 |
| Tannins (m/g GAE) | 5.862±0.01 | 2.100±0.00 |
| Alkaloids (mg/g) | 13.66±0.03 | 10.30±0.03 |
| Flavonoids (mg/g RE) | 97.259±0.26 | 2.741±0.02 |
| Saponins (%) | 1.34±0.01 | 2.04±0.04 |

Table 2: Growth performance and survival of *H. longifilis* fed experimental diet for 70 days.

| Parameter | AL1 | AL2 | AL3 | AL4 | AL5 |
|---|---|---|---|---|---|
| Initial weight (g) | 3.70±0.12 | 3.87±0.07 | 3.90±0.06 | 3.80±0.05 | 3.83±0.03 |
| Final weight (g) | 25.76±1.33$^{a}$ | 18.00±0.58$^{b}$ | 19.33±0.88$^{b}$ | 24.00±3.46$^{a}$ | 25.67±1.76$^{a}$ |
| Feed intake (g) | 33.96±0.64$^{a}$ | 28.20±0.69$^{b}$ | 29.41±3.76$^{b}$ | 31.43±2.41$^{a}$ | 33.61±1.45$^{a}$ |
| Weight gain (g) | 22.06±1.42$^{a}$ | 14.13±0.52$^{b}$ | 15.46±0.85$^{b}$ | 20.20±3.46$^{a}$ | 21.87±1.51$^{a}$ |
| SGR (%g/day) | 2.77±0.04$^{a}$ | 2.19±0.04$^{b}$ | 2.28±0.04$^{b}$ | 2.63±0.08$^{a}$ | 2.72±0.03$^{a}$ |
| FCR | 1.53±0.05$^{c}$ | 1.99±0.43$^{a}$ | 1.90±0.48$^{b}$ | 1.55±0.22$^{c}$ | 1.51±0.04$^{c}$ |
| PER (%) | 1.62±0.00$^{a}$ | 1.25±0.01$^{b}$ | 1.32±0.01$^{b}$ | 1.60±0.02$^{a}$ | 1.64±0.00$^{a}$ |
| Survival rate (%) | 90.00±7.64$^{a}$ | 85.00±2.89$^{ab}$ | 83.67±21.28$^{b}$ | 90.33±4.41$^{a}$ | 95.00±2.89$^{a}$ |

$^{abc}$Means on the same row with different superscripts are significantly (P<0.05) different *FCR, feed conversion ratio, PER, protein efficiency ratio What of SGR*

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# DIVERSITY AND ANTIBIOTIC RESISTANCE OF POTENTIAL *Vibrio* PATHOGENS – CASE STUDY OF MEDITERRANEI AND ORIENTALIS CLADES IN THE EASTERN ADRIATIC BIVALVE AQUACULTURE

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**Introduction**

Bivalves, which filter seawater as part of their feeding process, are particularly prone to bacterial colonization of their tissues. Among the bacteria commonly found in marine environments, *Vibrio* species are predominant and include commensals, opportunistic pathogens, and significant pathogens of both marine organisms and humans. Human infections are most frequently associated with the consumption of contaminated seafood, especially bivalves, which are often eaten raw or undercooked. In addition to the threat they pose to human health, *Vibrio* species can cause serious diseases in bivalves, impacting aquaculture production. Moreover, studies indicate that rising of seawater temperatures associated with global warming will significantly affect the dynamics of *Vibrio* genus species. Therefore, understanding the structure of natural *Vibrio* communities, including the presence of potential pathogens, is crucial for the management of bivalve aquaculture. Species belonging to the Mediterranei and Orientalis clades, such as *V. mediterranei*, *V. bivalvicida* and *V. tubiashii*, have frequently been associated with mortality events in marine organisms, but are under investigated in the context of eastern Adriatic bivalve aquaculture. In addition, the increasing problem of antibiotic resistance, driven by the overuse of antibiotics and anthropogenic pollution in marine environments, represents a growing challenge. Despite its importance, antibiotic resistance among *Vibrio* species in the eastern Adriatic bivalve aquaculture remains poorly investigated when it comes to Mediterranei and Orientalis clade species.

**Materials and methods**

Our study is focused in two protected marine reserves – Lim Bay and Mali Ston Bay, locations which are known for aquaculture of Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819 and *Ostrea edulis* Linnaeus, 1758. On both location we sampled seawater, sediment and bivalve tissue bimonthly over the course of one year. All bacteria isolated on *Vibrio* selective media were screened with MALDI-TOF mass spectrometry to confirm the genus and clade. More precise species identification was carried out by sequencing pyrH gene for improved taxonomic resolution. All isolates identified as members of Mediterranei and Orientalis clade were tested for antibiotic resistance *via* disk diffusion on Mueller Hinton agar for 12 selected antibiotics which are frequently used in aquaculture and medicine.

**Results**

MALDI-TOF MS results showed that 58 isolates belonged to Mediterranei clade while only 21 Orientalis clade isolates were found. Isolates of both clades were found during warmer periods especially in Lim Bay at seawater temperatures above 17°C. Furthermore, Orientalis clade isolates didn't show a high incidence of antibiotic resistance except for erythromycin (42.9%). For Mediterranei clade, higher occurrence of resistance to erythromycin (62.1%) was found. Also, while only 15.5% of isolates were resistant to oxytetracycline, 58.6% were intermediately resistant. Almost 71% of isolates were multi-resistant, meaning that they were resistant to two or more antibiotics.

This study provides the first report on the diversity and antibiotic resistance of *Vibrio* species from the Mediterranei and Orientalis clades associated with bivalve aquaculture in the eastern Adriatic. The findings highlight the need to focus on a broader range of species that make up the *Vibrio* community, in order to improve the current knowledge of the risk for infections and diseases outbreaks in bivalve aquaculture.

This will be crucial in the near future, considering the predictions that increasing anthropogenic pressure and climate change will enhance the susceptibility of farmed bivalves to potentially pathogenic *Vibrio* species and promote the spread of antibiotic-resistant strains, posing a growing threat to the sustainability of bivalve aquaculture.

**Acknowledgements**

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# A NEW METHOD TO CLASSIFY FERTILIZED EGGS IN AQUACULTURE

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**Introduction**
Early mortality is a persistent issue for marine aquaculture hatcheries; thus, quality control during this phase is -stage vital to enhance survival rates and overall production. Fertilized egg quality is the first point to be checked so that it is very important to culture only the best batches and to discard them if quality is not good.

This study explores a novel approach to classify fish eggs based on visual characteristics using automation and AI, which has the potential to improve hatchery quality assurance.

The efficacy of this new method was evaluated using bluefin tuna (*Thunnus thynnus*) and sea bream (*Sparus aurata*) eggs under experimental conditions at the IEO-ICAR infrastructure in Murcia, Spain. The impact on egg viability, hatching rates, and operational processing speed were all assessed.

**Materials and methods**
The EggSorter is a new mechanical and fluidic system which can automatically grab individual eggs from an inlet reservoir, analyse each one of them thanks to an optimised optical system and classify the eggs into various categories using image processing and artificial intelligence algorithms. Following analysis, the eggs are directed into one of two fluidic paths according to the analysis criteria.

An artificial intelligence–based image analysis pipeline was developed to classify bluefin tuna and sea bream eggs based on the quality and stage of development. High-resolution brightfield images were captured by the EggSorter and annotated by the team at IEO-ICAR. For sea bream, an algorithm was developed to distinguish gastrula-stage eggs of good quality from poor quality, dead, or at another developmental stage eggs. The training dataset consisted of 3657 images, with 738 images labelled as ‘good’ (good quality eggs at gastrula stage) and 2919 images labelled as ‘bad’ (poor quality eggs, dead eggs or eggs at a different developmental stage than gastrula). For bluefin tuna, the training dataset consisted of 686 images, with 126 images labelled as ‘good’ (good quality eggs) and 560 images labelled as ‘bad’ (poor quality eggs or dead eggs).

9 different batches of fertilized sea bream coming from broodstock kept in tanks in IEO-ICAR and 7 different batches of bluefin tuna eggs coming from both, a broodstock kept in tanks in IEO-ICAR and commercial floating net cages off the Cartagena coast, El Gorguel SE Spain, owned by Ricardo Fuentes e Hijos, were used. Eggs coming from cages were transported early morning to the Spanish Institute of Oceanography (IEO), Planta Experimental de Cultivos Marinos (Puerto de Mazarrón, Murcia, Spain), and they arrived in morula stage. All the fertilized eggs were cleaning and placed to incubate until the gastrula stage. Then some eggs were separated as control and incubated in standard conditions until 12 hours after hatching in three small glasses (250 ml sea water, 25 eggs per glass and temperature control at 21±1ºC for sea bream and 24±1ºC for bluefin tuna) and remaining eggs were processed by the EggSorter.

After processing, egg classified as good quality eggs were incubated in the same standard conditions than above. 12 hours after final hatching, all the glasses were counted, and dead eggs, alive larvae and dead or folded larvae were determined). Hatching rate (100 x hatched larvae/fertilized eggs incubated) and Deformity rate (100 x folded or dead larvae/total hatched larvae)

Results
The efficacy of this new method was evaluated using bluefin tuna (*Thunnus thynnus*) and sea bream (*Sparus aurata*) eggs under experimental conditions at the IEO-ICAR infrastructure in Murcia, Spain. The EggSorter is able to process up to 1,300 eggs/hour, separating good and poor quality eggs. The impact on egg viability, hatching rates, and operational processing speed were all assessed. Results demonstrated no significant adverse effects on hatching rates or larval normality when comparing sorted and control groups. In bluefin tuna, automatically sorted eggs showed a slightly higher average hatching rate (88.5%) compared to controls (83.8%), with comparable levels of larval normality.

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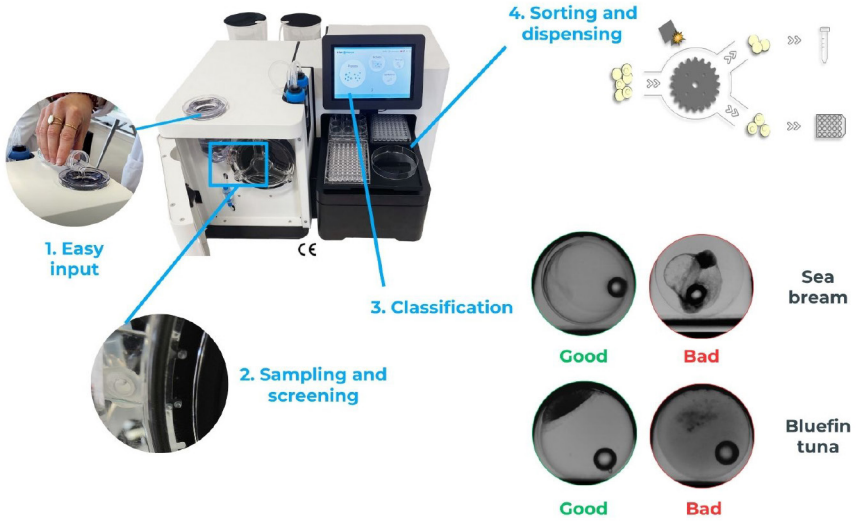


| **ABFT** | Hatching rate % | | Normal larvae % | |
|---|---|---|---|---|
| | Control | Egg Sorter | Control | Egg Sorter |
| Average÷std | 83,82 ± 14,19 | 88,54 ± 10,37 | 87,10 ± 10,60 | 83,71 ± 8,6 |
| **Sea Bream** | Hatching rate % | | Normal larvae % | |
| | Control | Egg Sorter | Control | Egg Sorter |
| Average÷std | 74,30 ± 11.68 | 67.43 ± 6.56 | 84.57 ± 9.79 | 76.86 ± 5.72 |

**Conclusions**

This method shows great potential as a research and broodstock management tool. With further optimization—particularly in processing speed, but also in the number of data of the algorithm —it could become a valuable asset for early embryonic screening, helping hatcheries improve stock quality and reduce early-stage mortality.

**Acknowledgements**

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# TRANSCRIPTOME ANALYSIS OF RED BLOOD CELLS IN RESPONSE TO THE RED SPOTTED GROUPER NERVOUS NECROSIS VIRUS (RGNNV)

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Red spotted grouper nervous necrosis virus (RGNNV) is the most widespread betanodavirus strain affecting numerous fish species, with European seabass (*Dicentrarchus labrax*) being highly susceptible. RGNNV causes up to 100% mortality in larvae and juveniles and significant losses in adult fish by targeting central nervous tissue, particularly the retina and brain.

In order to develop effective vaccines, the host immune response to the pathogen should be fully understood. However, in the infection produced by RGNNV in European sea bass, the role of RBCs is unknown. Nucleated RBCs are known to act as phagocytes, antigen-presenting cells, and modulate leukocyte activity, producing cytokines and other signaling molecules in response to viral infections (1). The aim of this work was to study the participation of European sea bass RBCs in the immune response to RGNNV infection.

European sea bass purified RBCs were exposed *ex vivo* to RGNNV, and the expression of genes involved in the antiviral immune response was evaluated. On the other hand, *in vivo* assays were performed in which juvenile individuals were infected with RGNNV and at 1- and 3-days post infection brain and purified RBCs from peripheral blood samples were used to study gene expression by RT-qPCR. In addition, RBCs transcriptome was also evaluated by means of RNA-sequencing.

The *ex vivo* results showed that RGNNV was able to stimulate the expression of some genes related to the antiviral immune response in RBCs exposed to RGNNV, such as *isg15* (an interferon-induced protein that has been implicated as a central player in the host antiviral response). The *in vivo* results showed that RGNNV was able to stimulate the expression of *mx* (a GTPase that is part of the antiviral response and is known to inhibit viral replication) in RBCs, both at 1- and 3-days post-infection. It is noteworthy that *mx* was also highly expressed in the brain, the target organ of RGNNV infection. The RNA-sequencing results showed over-representation of antiviral immune response pathways in RBCs from RGNNV infected European sea bass, depicting a clear response to the virus, mediated by cytokines and antiviral proteins.

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# RED BLOOD CELLS RESPONSE TO RECOMBINANT MODULAR NANOPARTICLE-BASED VACCINE AGAINST IPNV INFECTION

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Infectious pancreatic necrosis virus (IPNV) remains a critical challenge in salmonid aquaculture, with conventional vaccines often presenting limited immunogenicity and labor-intensive production (Rimstad 2014). Recognizing that fish nucleated red blood cells (RBCs) are not only the most abundant cells in circulation but also have emerged as pivotal immune actors—capable of antigen processing, presentation, and cytokine production—we developed a recombinant, modular nanostructured vaccine (nanopellets, NPs) to target this cell type as ideal carriers for systemic vaccine delivery (Nombela and Ortega-Villaizan 2018, Puente-Marin, Nombela et al. 2018). These NPs consist of the IPNV-VP2 capsid protein, either alone (IPNV$^{NP}$) or fused to rainbow trout interferon gamma (IFNγ) to generate an adjuvanted construct (IPNV-IFNγ$^{NP}$), while a fluorescent iRFP$^{NP}$ served as a control. NP constructs were expressed in E. coli and characterized by electron microscopy, which confirmed their stable nanostructured assembly under extreme conditions relevant for aquaculture.

In vitro assays with purified rainbow trout RBCs demonstrated that IPNV-IFNγ$^{NP}$ were efficiently internalized by RBCs in a dose- and time-dependent manner, outperforming non-adjuvanted counterparts. Intraperitoneal administration of IPNV-IFNγ$^{NP}$ led to enhanced presence in blood as well as in primary immune organs such as the head kidney and spleen. RNA sequencing (RNA-seq) analysis identified over 1,800 differentially expressed genes in IPNV-IFNγ$^{NP}$-treated RBCs that were enriched in interferon signaling, vesicle trafficking, and mitochondrial pathways. Also, the transcriptomic profiling of RBCs from immunized fish revealed the robust upregulation of antiviral genes (e.g., *mx*, *vig1*, *isg15*, and *ifit5*).

These results underscore the potential of harnessing RBCs as systemic antigen carriers to enhance innate and adaptive immune responses. Further, NP-based subunit vaccine is a promising strategy to promote more sustainable and welfare-friendly aquaculture practices. Ongoing work will focus on functionalizing the NP-based platform with ligands targeting RBCs to improve immunization strategies.

**Acknowledgements:**
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# CLOT FORMATION ASSAY (CFA): A NOVEL METHOD FOR ASSESSING PLASMA CLOTTING CAPACITY IN GILTHEAD SEABREAM (*Sparus aurata)*

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## Introduction

Aquaculture has grown in production and economic importance worldwide. Climate change is expected to increase disease outbreaks in farmed fish, highlighting the need for improved diagnostics. Standardised, non-lethal health monitoring methods are essential. While human haematology is well established for disease diagnosis, fish haematology is gaining recognition as a biomarker system to detect physiological changes caused by nutritional deficiencies, environmental stress, toxins and diseases. This study focuses on the development of a new standardised assay to correlate coagulation parameters with the health status of sea bream (*Sparus aurata*), providing a tool for improved health assessment in aquaculture systems [1].

## Materials and methods

To standardize the assay, it was necessary to determine the concentration of both, sodium citrate, required to prevent coagulation in sea bream blood, and calcium chloride, required to initiate the reaction. Seabream blood samples (250 $\mu$L) were collected in 1.5 mL Eppendorf tubes containing sodium citrate and kept on ice. Sodium citrate concentrations ranging from 3.8 to 20 % were tested. The samples were then centrifuged at 2500 g for 15 min to obtain cell-free plasma (CFP), which could subsequently be stored at -40 °C. In 96-well, clear, flat-bottomed, untreated plates, 150 $\mu$L of 0.9% Braun saline, 40 $\mu$L of CFP and 10 $\mu$L of calcium chloride were added to each well. Calcium chloride concentrations were evaluated from 0 to 350 mM. Finally, absorbance was recorded at 405 nm every minute for 1 hour. Once optimized the assay, the evaluation of the coagulation of fish exposed to chitosan with Mw 20 kDa and degree of acetylation of 60 % for 1 h was performed. An untreated fish group was used as control. Blood samples were taken 8 h after the exposition to chitosan.

## Results

To prevent and induce coagulation, respectively, 15 % sodium citrate and 200 mM calcium chloride were selected as the best salt concentrations for the developed procedure. The analysis of the fish blood coagulation after the exposure to chitosan revealed significant differences in all parameters analysed (Fig. 1), with the experimental group unable to coagulate under the same conditions as the control group (Fig. 2).

## Discussion/conclusions

The methodology developed is a complement for the evaluation of seabream immunity, as plasma procoagulant activity is related to acute stress and potential death from spontaneous thrombi caused by elevated adrenaline in the blood. In teleosts, hypocoagulant capacity correlates with liver damage due to a fall in the concentration of proteins that belong to the coagulation cascade. Nevertheless, when hypocoagulability exists in the absence of liver damage, the compounds or conditions tested may act as protective agents against thrombosis. To our knowledge, the effect of chitosan on fish blood coagulation has never been evaluated before. Further analysis will allow us to determine whether chitosan could serve as a protective compound against thrombus generation [1,2].

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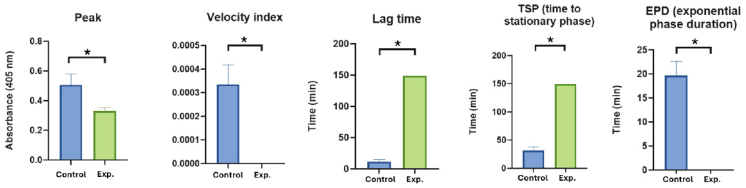


Fig. 1. Peak (maximum absorbance), Velocity index ((ΔAbsmax)/(Δt)), Lag time (time required for coagulation to start), TSP (time to stationary phase), EPD (exponential phase duration). Statistical significance was determined using Student's t-test (*, $p \leq 0.05$).

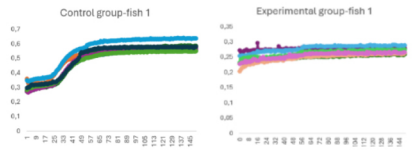


Fig. 2. Coagulation curves of representative fish from the control (left) and experimental (right) groups. Y-axis: absorbance; x-axis: time. The different colour curves show different replicates.

Acknowldegements

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# CLIMATE-DRIVEN SHIFTS IN WATER COLUMN STRATIFICATION AND THEIR EFFECTS ON MEDITERRANEAN FINFISH FARMING

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**Introduction**

Due to the increase of $CO_2$ emissions and other greenhouse gases, the oceans are experiencing warming caused by the global phenomenon known as climate change. This effect is especially evident in enclosed seas such as the Mediterranean. In this context, marine heat waves have become an increasingly frequent and intense phenomenon, seriously affecting the stability of marine ecosystems. In this context of climate change, activities such as marine aquaculture are subject to multiple stress factors such as those exerted by global climate change, an effect that affects the physiology, development and growth of fish species.

**Material and Methods**

Using data from Copernicus Marine Services, this study examines the effects of climate change on the thermal structure of the water column along a latitudinal and longitudinal gradient in the Mediterranean. The research focuses on 12 marine aquaculture sites in different countries (Spain, Tunisia, Greece, Croatia, Turkey, Cyprus and Egypt). Specifically, it analyzes seasonality over two periods (a period before and a period after relevant effects of climate change), as well as the evaluation of thermal anomalies during the summer season. To determine the sustainability of marine aquaculture systems throughout the Mediterranean, a climate risk indicator using R Studio will be applied to these locations, together with the interpretation of water column graphs.

**Results and Conclusions**

Thanks to the study of the thermal stratification in the water column, the deepening of the 25º C isotherm influenced by latitude and longitude is detected. The study of two different periods helps to find differences in the deepening of the isotherm, where warm water masses reach greater depths in the period more influenced by climate change, as well as their increasingly earlier appearance in the summer period (Figure 1) (Table 1). The locations of Guardamar (Spain), Ionian Islands (Greece) and Limassol (Cyprus) take the highest values of isotherm deepening in the study period following global warming, with values of 14, 16 and 19 depth-month units, respectively. Specifically, locations located in the eastern Mediterranean area have a higher number of depth anomalies in the summer period. In addition, temperature-based climate risk indices indicate that locations in this area indicate high risk (values greater than 0.7-0.8) in terms of the sustainability of the aquaculture season and potential impacts, such as the Nile River (Egypt) and Limassol (Cyprus).

The continuous increase in mean ocean temperature and the intensification of marine heat waves could significantly affect European aquaculture, as heat stress compromises production. Thermal stratification limits the circulation of nutrients and oxygen, affecting the health and productivity of aquatic crops. Therefore, the sector must adapt to changes in marine ecosystem services to maintain sustainable production. It is crucial to develop climate change management and mitigation strategies in the Mediterranean region in order to have the tools to face these challenges and maintain optimal conditions in aquaculture systems.

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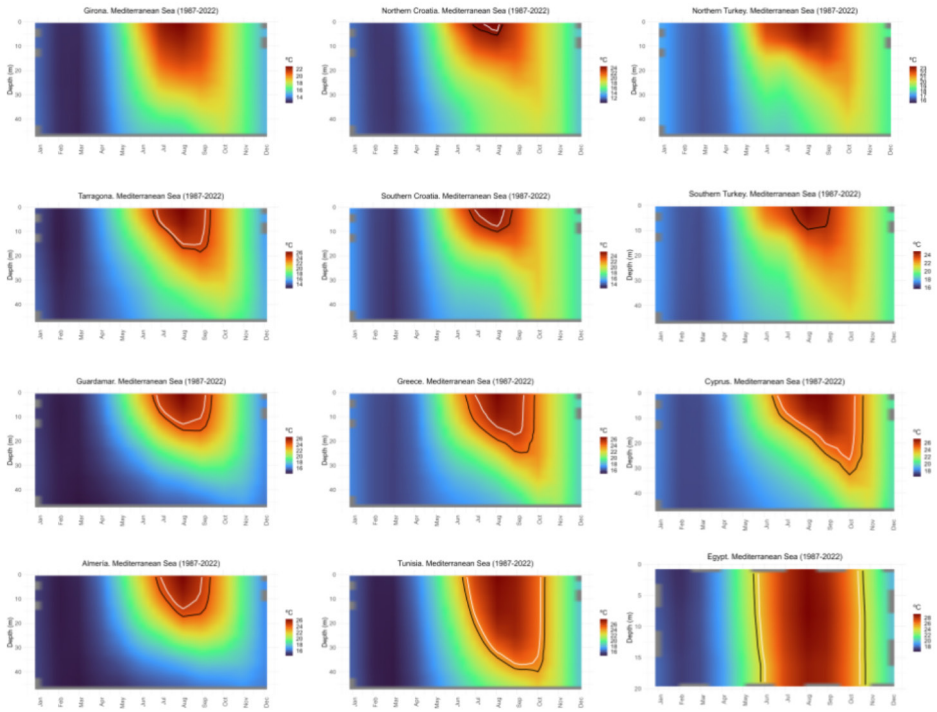


*Figure 1*. Depth of the 25º C isotherm in the 12 study localities during the summer period. Graphs obtained thanks to R-Studio.

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## Acknowledgments

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# DIGESTIVE ENZYME EVALUATION IN MULLET (*Liza aurata)* UNDER SEVERAL SUSTAINABLE DIETS BASED ON BIOFLOC MEAL

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## Introduction

Finding alternatives to fishmeal is essential for sustainable aquaculture. Biofloc technology is a system that promotes the growth of beneficial microbial communities, which convert waste nutrients into protein biomass, improving water quality and providing a source of food. Biofloc meal, obtained by harvesting and processing aggregates from biofloc systems could be used as a sustainable ingredient for aquafeeds. Moreover, diversification in aquaculture is essential to enhance resilience, reduce economic risks and promote environmental sustainability. In the last years, mullet (*Liza aurata*) has gained a solid foothold in the market, driven by its outstanding nutritional quality. The present study aims to determine the optimal replacement level of fishmeal with biofloc meal as alternative protein source to improve the culture of mullet.

## Material and methods

Three experimental diets were formulated (35% P, 8% L and 40% CH): control diet based on fishmeal and two experimental diets with fish meal replacement by biofloc meal at 20 and 30% levels (C, B20 and B30, respectively). Mullets (*Liza aurata*) were randomly distributed in 9 tanks, each containing 12 fish (initial weigh 112 ± 1g) in 350L tanks with brackish water of 25.0 ± 0.2‰ of salinity and 25.8 ± 0.3ºC of temperature and maintained under a Recirculating Aquaculture System. Fish were manually fed *ad libitum* twice a day, six days a week. After 80 days, 9 fish of each experimental group were sampled and digestive tract excised and divided into pyloric caeca, anterior and posterior intestine. After processing, trypsin and chymotrypsin were determined by Lazo *et al.* (2000) and Faulk *et al*. (2007). α-amylase and lipase were determined by commercial kits (Spinreact, Ref. 41202 and Ref.1001275). Protein concentration was determined by Bradford method. Results are presented as mean ± SEM. Two-way ANOVA was used, with diets and digestive section as fixed factors. When interaction was significant, diet and section effects were analyzed by a one-way ANOVA. F values were significant for $P<0.05$ and differences were determined following a post-hoc analysis using Tukey test. Statistical analyses were performed with SPSS v.25.

## Results

Results for activity of the digestive enzymes assayed are presented in Figure 1. Regarding sections of digestive tract, anterior intestine was the section with significantly highest activity for all enzymes independently of treatment, except for lipase, in which non-significant differences were detected. As for the effect of the dietary treatment, chymotrypsin, lipase, and amylase, except in the pyloric caeca where no significant differences, presented the highest values ($p < 0.05$) in the B30 experimental group, both in the anterior and posterior intestine. In contrast, the B30 group showed significantly lower values in the case of trypsin.

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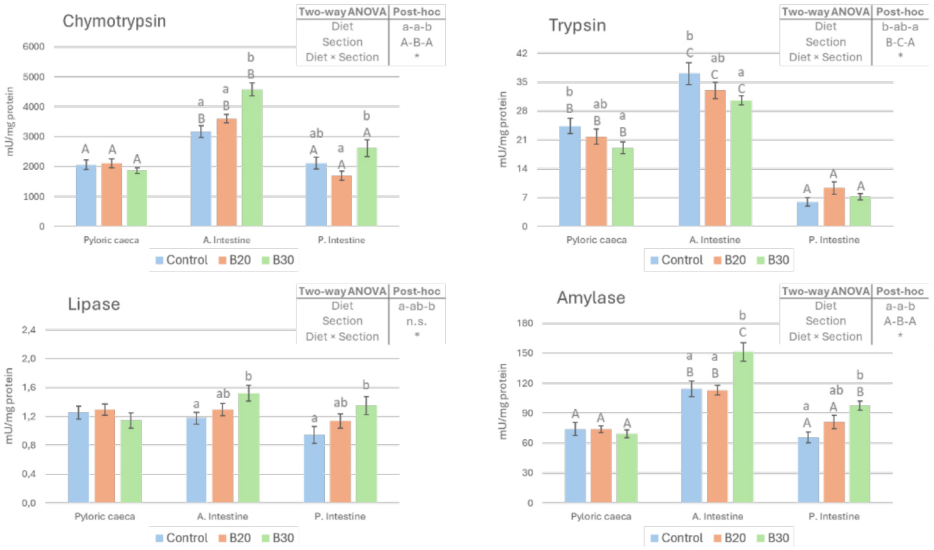


**Fig.1.** Chymotrypsin, trypsin, lipase and amylase specific activities in pyloric caeca, anterior and posterior intestine of *Liza aurata*. Values are means ± SEM (n=9). For two-way ANOVA, letters indicate influence (P<0.05) of experimental diets and asterisks interaction among factors. When interaction was detected, one-way ANOVA was performed. Over the bars, small letters indicate differences (P<0.05) induced by experimental diet in each digestive section. Capital letters indicate differences (P<0.05) among digestive section in each experimental diet.

## Discussion

In fish, pyloric caeca and anterior intestine are the main site of secretion for pancreatic enzymes such as lipase, amylase, and alkaline proteases. Moreover, these parts of the digestive tract are also the sections in which nutrient absorption predominantly occurs, with the posterior intestine contributing to a lesser extent. In the present study, results show that in mullet, activities of chymotrypsin, trypsin and amylase were generally higher in the anterior intestine and, to a lesser degree, in the pyloric caeca and posterior intestine. Dietary changes can affect digestive enzyme activity, and in this study, fish fed a diet containing 30% biofloc meal showed higher activity for all enzymes determined except trypsin. Since macronutrient levels were the same across experimental diets, this improvement could be related to biofloc properties. In this sense, biofloc is composed of microorganisms (bacteria, microalgae, protozoa) and organic matter that might stimulate endogenous enzyme secretion, provide exogenous enzymes, and modulate intestinal microbiota, thereby supporting gut health. Similarly, Ekasari et al. (2019) reported that 10 and 20% biofloc meal dietary inclusion enhanced growth, feed utilization, and health in African catfish (*Clarias gariepinus*). In conclusion, replacing 30% of dietary fishmeal with biofloc meal in mullet may support or even improve an optimal digestive activity.

## Acknowledgements

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# EVALUATING REGIONAL GOVERNANCE IN NORWEGIAN AQUACULTURE: A 15-YEAR REVIEW OF COUNTY-LEVEL ADMINISTRATION POST-REFORM

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In 2010, Norway implemented a governance reform transferring aquaculture licensing and site coordination responsibilities from the Directorate of Fisheries to county municipalities (fylkeskommuner). The reform aimed to strengthen regional governance by decentralizing aquaculture management, improving coordination, enhancing regional development, and enabling more holistic coastal zone planning. It also introduced statutory deadlines for processing aquaculture site applications to improve efficiency and predictability for the industry.

This study evaluates the outcomes of that reform 15 years later, focusing on administrative performance, coordination, and regional development impacts.

Using a mixed-methods approach—including 38 stakeholder interviews, document analysis, and statistical review of 1,200+ site applications (2020–2023)—we assessed the counties' delivery capacity, inter-agency collaboration, and effects on the aquaculture sector.

**Key findings**
Counties were well-prepared for their new responsibilities, aided by staff transfers from the Directorate of Fisheries and early investments in capacity-building. Over time, counties developed strong professional environments, particularly through the establishment of the FAKS network (translated: Counties' Aquaculture Cooperation), which has been instrumental in promoting coordination, knowledge sharing, and standardization.

While counties generally meet statutory deadlines for standard applications, overall processing times have increased from 2020 to 2023, due to growing complexity of applications. Also, delays were often due to bottlenecks at other authorities, especially the County Governor (Statsforvalteren) and the Norwegian Food Safety Authority (Mattilsynet).

Findings show that counties have largely succeeded in managing their new responsibilities. The counties have effectively coordinated the multi-agency site approval process and are seen as accessible, competent, and service-oriented. However, challenges remain in ensuring consistent interpretations of what constitutes a complete application and in managing overlapping applications for the same sites. Also, challenges remain in balancing environmental and industry interests.

Counties have leveraged their broader responsibilities in planning, education, and business development to support the aquaculture sector. This includes initiating inter-municipal planning processes, funding knowledge-building projects, support for marine education, and facilitating dialogue between stakeholders.

However, increasing complexity of applications, increasing competition for coastal space and the emergence of new species (e.g., cod) demand improved tools for cumulative impact assessment and stronger cross-sectoral governance.

We conclude that while the reform has strengthened regional governance and service delivery, future success depends on continued investment in coordination mechanisms, digital infrastructure, and adaptive regulatory frameworks.

# EFFECT OF TRANSFER PROCESSES ON CONDITIONS OF JUVENILE PIKEPERCH (*Sander lucioperca*) CULTURED IN RAS: ACCLIMATIZATION AND STRESS ELIMINATION

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## Introduction

Pikeperch is a well-appreciated freshwater fish, due to its nutritional value and palatability (Péter et al, 2023). The rearing of these species in Recirculating Aquaculture System (RAS) is not optimized due to the maintenance of high-water quality and high fish stress levels, which causes an overall decrease in production (Thomas et al., 2020). It has been reported that pikeperch is susceptible to changing conditions, given its high basal cortisol levels, and the duration of its recovery period (Baekelandt et al., 2019; Pourhosein-Sarameh et al., 2013; Pourhosein-Sarameh & Falahatkar, 2024).

The transfer from one tank to another is a source of stress and a necessary step duringthe rearing of this species. To minimize the stress caused by this process, assessing the shortest acclimatation time is key. Therefore, the aim of this study was to test the effect of transferring two sizes of juvenile pikeperch into different tanks on length of acclimatation period, survival, growth, oxidative stress, blood biochemistry to identify the optimal juvenile size to perform the transfer.

## Materials and methods

In total, 2700 juveniles for each group as: on-growing with total length (TL) = 161.4 ± 10.4 mm and body weight (BW) 27.2 ± 7.2 g, and early grown-out with TL = 242.1 ± 18.87 mm, BW = 104.1 ± 14.7 g were cultured 3 months before the transfer in three 1.5 $m^3$ tanks connected to an experimental 30 $m^3$ RAS. Three fish per tank were sacrificed and sampled one day before transfer. Fish were transferred manually using nets from three tanks to seven tanks connected with the same RAS.

Three samples per tank were collected immediately after the transfer (T), one day post transfer (1DPT), 7 days post transfer (7DPT), 21 days post transfer (21DPT), 35 days post transfer (35DPT) and 49 days post transfer (49DPT). Samples of blood, brain, gill, liver and kidney were taken for evaluation of blood profile and biochemisry oxidative stress and antioxidant enzymes. The required time for acclimation of each category was assessed by the comparison of founded values with optimal values of each parameter for pikeperch which were mainly published by Kolářová and Velíšek (2012). This procedure was repeated twice for each experimental group.

## Results

Cortisol, glucose, lactate and ammonia presented significantly higher concentrations until 35DPT for on-growing and until 7DPT for grown-out. On-growing individuals required 35 days to eliminate oxidative stress in brain, gill, liver and kidney, in contrast to grown-out group, which took 7 days.

Larger individuals exhibit equivalent levels of stress sensitivity yet demonstrate a more abbreviated acclimatization phase. This suggests that the optimal timing of the transfer process can be determined through strategic selection.

## Discussion

Previous results (Baekelandt et al., 2019; Ende et al., 2021; Malinovskyi et al., 2020), aligns with the fact that pikeperch is a highly sensitive species. (Kozłowski & Piotrowska, 2024; Pourhosein-Sarameh & Falahatkar, 2024). Size seems to be a factor that infuences in the acclimatization period. Recent research has further established differences in stress response between wild and pond-reared pikeprch juvenile (W = 16g) during transfer (Péter et al., 2023). Other parameters such as domestication process of the broodstock, life cycle and individual physiology influences acclimatization period (Falahatkar et al., 2025).

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**Acknowledgements**
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# EXPLORING TRANSFORMATIVE SOCIAL INNOVATIONS: INSIGHTS FROM CROSS-FIELD DIALOGUE AND APPLICATION IN SICILY'S AQUATIC FOOD SYSTEMS

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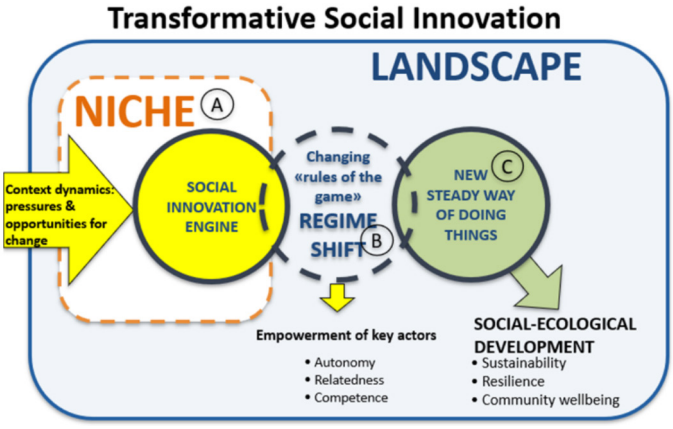
***Background and aim***
Food systems require systemic change, yet how transformative processes unfold is not always clear. Social innovations, if properly embedded, can generate bottom-up solutions that stabilise new socio-environmental trajectories. This study investigates how a diffuse Living Lab in Sicily (Innovittica, Trapani) mobilises niche experiments, governance structures, and circular bioeconomy practices to transform "waste" into resources and provide new opportunities for coastal fisheries and SMEs.

***Methods***
Semi-structured interviews were conducted with fishers, SMEs, authorities, CSOs and academics, alongside Living Lab observation and document review. Analysis was structured along three transition levels: (i) niche-level innovations, (ii) regime changes, and (iii) stabilisation pathways for improved socio-environmental contexts.

***Results***
Key transformations were observed across three domains. ***Doing***: new products and processes were co-designed and validated through product valorisation and sensory analysis. ***Knowing***: hands-on co-creation workshops and continuous feedback loops facilitated stakeholder learning. ***Governing***: bottom-linked governance via a Temporary Association (ATS), multi-actor roundtables and transparent facilitation mechanisms ensured shared decision-making.

***Discussion and conclusion***
Our study illustrates how transformative social innovation can stabilise a new, equitable way of doing by reframing side-streams as assets, embedding by-product valorisation in policy, and formalising collaborative governance as a regional platform. These processes demonstrate the complementarity of niche experimentation and bottom-linked governance in fostering sustainable and socially just aquatic food systems.



# ENHANCING ATLANTIC SALMON RESILLIENCE THROUGH FUNCTIONAL FEEDS: THE ROLE OF FUCOIDAN IN IMMUNOMODULATION AND DISEASE MITIGATION

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**Introduction**

Norwegian salmon farming is a vital economic sector but faces significant challenges due to high fish mortality rates, particularly during the seawater (SW) phase. For instance, winter ulcers caused by bacteria such as *Moritella viscosa* or *Tenacibaculum* sp. pose a major threat. Functional feeds containing bioactive compounds, such as fucoidan from sugar kelp (*Saccharina latissima*) offer a potential solution to improve fish resilience. The RESILIENT SALMON project (Research Council of Norway – 294821) investigates how fucoidan can enhance immune responses and reduce susceptibility to diseases.

**Materials and methods**

Fucoidan was extracted from sugar kelp using a factorial design to optimize key parameters, such as temperature, acid concentration and time, in a pilot biorefinery process. Bioactivity test in salmon kidney cells (SHK-1) determined the most effective fucoidan fraction for immune stimulation, based on sulfation levels, yield and immunostimulatory effects (Michalak et al 2024). Then, the most promising fraction was upscaled for evaluation in functional diets for Atlantic salmon (*Salmo salar*).

Experiment 1 (Exp.1): After an *in vitro* evaluation in SHK-1 cells, the immunomodulatory effects of fucoidan in pre-smolt Atlantic salmon were tested in freshwater (FW) phase. Fish were fed experimental diets containing 0% (negative control), 0.1%, 0.2%, or 0.4% fucoidan, or a positive control diet containing 0.2% MacroGard®. Distal intestine (DI) samples were collected for microbiota characterization (by 16S rRNA-seq) and detection of immune-related biomarkers (using RNAseq, RT-qPCR, immunohistochemistry and proteomics). Also, primary cultures of head kidney leukocytes (HKLs) were obtained from fish fed experimental diets and stimulated with proteins from *Aeromonas salmonicida*. Then, the protein level of cytokines such as TNF-α and IL-10, as well as effector molecules (i.e., iNOS and HSP70) were detected by indirect ELISA.

Experiment 2 (Exp. 2): We evaluated whether feeding a diet with 0.2% fucoidan for four weeks post-vaccination in FW could enhance the immune response of Atlantic salmon exposed to inactivated *Tenacibaculum maritimum* (via intraperitoneal injection) after SW transfer. For this, immune biomarkers in the DI and head kidney (HK) were analyzed by RT-qPCR and indirect ELISA at the end of the FW stage, and before and after stimulation in SW.

Experiment 3 (Exp. 3): investigate the impact of feeding a 0.2% fucoidan diet on the resistance of Atlantic salmon to *Tenacibaculum dicentrarchi*. Fish were fed the fucoidan-supplemented diet for four weeks in FW, followed by four weeks on a control diet in FW. Then, fish were transferred to saltwater and challenged with *T. dicentrarchi*. The control group, which only received the control diet throughout the experimental period, was also infected with *T. dicentrarchi*. We assessed mortality rates and immune responses to evaluate the mode of action underlying the observed health effect of the functional diet containing fucoidan.

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**Results and Discussion**

In Exp.1, fucoidan enhanced immune and antioxidant responses in Atlantic salmon. For instance by upregulating expression of *sod-1*and *prx* in SHK-1 (at 72h). Also, HKLs from fish fed 0.2% fucoidan or MacroGard® and stimulated with proteins from *A. salmonicida* had increased production of TNF-α (pro-inflammatory cytokine) and HSP70 (chaperone). In contrast, HKLs from fish fed 0.4% fucoidan had an increased level of IL-10 (anti-inflammatory cytokine). Feeding the 0.2% fucoidan diet also increased the presence of *Bacillus sp* in DI and up-regulated pathways related to immune cell development, tissue repair, and cell adhesion. At protein level, fish fed 0.2% fucoidan showed a higher production of MHC II, CD3, along with the up-regulation of *gata3*, suggesting an enhanced adaptive immunity. By proteomics in DI, we detected higher levels of immune-related proteins (gap junction and NOD-like receptor) and a lower level of proteins associated with amino acid metabolism and fatty acids degradation, indicating a metabolic shift towards immune resilience. These findings suggest that fucoidan, depending on dietary inclusion level, can enhance immune function, gut health, and overall resilience in salmon.

Results from Exp 2, showed that fucoidan supported growth and boosted immune responses when combined with a commercial vaccine. Fucoidan activated key immune-related genes in Atlantic salmon such as pro- and anti-inflammatory cytokines (*tnfa*, *il10*) in DI, while in the HK, an upregulation of antimicrobial peptides (i.e., *hepcidin*, *cathelicidin*) was detected after stimulation with inactivated *T. maritimum.* These effects were also confirmed at the protein level, reinforcing fucoidan's potential as a potent immune-modulating feed component.

Exp. 3 revealed that fucoidan significantly improved fish survival when exposed to the live *T. dicentrarchi*. Atlantic salmon fed fucoidan-supplemented diet showed a 51% higher probability of survival compared to the control group. Further, the levels of CD4-1+ T cells and IgM+ B cells in peripheral blood leukocytes (PBL) and the spleen increased as a result of fucoidan supplementation during the infection. Following the *T. dicentrarchi* infection, cytokine expression levels were also upregulated in the spleen and PBL of fucoidan-fed fish, suggesting that fucoidan may enhance T cell differentiation and immune activation.

**Conclusion**

Functional feeds enriched with fucoidan from sugar kelp, combined with nutritional programming, can significantly enhance salmon resilience and health beyond the specific vaccine effects, supporting the sustainable development of the aquaculture industry.

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# INVESTIGATION OF THE EFFECT OF HEAT STRESS ON EPIGENETIC MODIFICATIONS OF TESTES AND SPERM IN NILE TILAPIA *(Oreochromis niloticus)* AND ON ITS SPERM PARAMETERS

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**Introduction**
Heat stress represents a critical environmental factor that can significantly impair reproductive physiology in aquatic organisms such as Oreochromis niloticus. Chronic exposure to elevated temperatures disrupts spermatogenesis and leads to functional sperm damage. Epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNA activity, are known to play essential roles in regulating germ cell development and are sensitive to environmental fluctuations. Despite increasing interest in the relationship between epigenetic mechanisms and male fertility, integrated studies on how chronic heat exposure alters these regulatory layers in Nile tilapia remain limited. The objective of this study was to investigate how long-term thermal stress affects sperm epigenetic signatures and testicular histoarchitecture and to evaluate their association with sperm quality and reproductive success.

**Materials and Methods**
Experimental setup and animal husbandry: Sexually mature male Nile tilapia was randomly distributed into three groups, each exposed to a specific water temperature: the control group at 28 °C, the moderate stress group at 32 °C, and the high stress group at 36 °C. The fish were reared in aerated tanks under natural photoperiod conditions for a duration of 90 days. At the end of the experimental period, testicular and sperm samples were collected for subsequent analyses.

DNA methylation profiling by whole genome bisulfite sequencing: To evaluate global DNA methylation patterns, genomic DNA was extracted from sperm samples. The DNA was sheared to appropriate fragment sizes and ligated to methylated sequencing adapters. Following bisulfite treatment to convert unmethylated cytosines to uracil, libraries were amplified and sequenced using paired-end next-generation sequencing platforms. Raw sequencing reads were analyzed using the Bismark software pipeline, allowing alignment and methylation calling across CG, CHG, and CHH sequence contexts. Differentially methylated regions were identified between control and heat stress groups.

Expression analysis of small and long non-coding RNAs: Total RNA was isolated from spermatozoa using column-based purification kits. For small RNA analysis, libraries were constructed by ligating 3-prime and 5-prime adapters followed by reverse transcription and PCR amplification. For long non-coding RNA profiling, ribosomal RNA was selectively depleted before sequencing library preparation. High-throughput sequencing was performed on Illumina platforms. Sequencing data were aligned using HISAT2 for long reads and Bowtie2 for small RNAs. Transcripts were assembled using StringTie and differential expression was calculated using DESeq2.

Immunohistochemical detection of epigenetic and stress markers: Testis tissues were fixed in modified Davidson's solution to ensure optimal nuclear and cytoplasmic preservation. After fixation, tissues were embedded in paraffin, sectioned at five microns, and mounted on positively charged slides. Immunohistochemical staining was performed using primary antibodies specific to 5-methylcytosine as a marker of DNA methylation and histone modifications. Heat shock proteins Hsp70 and Hsp60, as well as the proliferation marker PCNA, were also evaluated. Sections were incubated with corresponding secondary antibodies and visualized using AEC chromogen. Counterstaining was performed with Gill's hematoxylin and slides were examined microscopically.

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Histopathological evaluation of testes: Paraffin-embedded testes fixed in modified Davidson's fixative were stained with hematoxylin and eosin. Tissue architecture was evaluated under light microscopy, focusing on the organization of seminiferous tubules, thickness of the germinal epithelium, presence of interstitial edema, and vacuolization. Particular emphasis was placed on identifying features associated with feminization such as disrupted spermatogenic layers and disorganized tubular structure.

**Results**

The 36°C group exhibited significant alterations across all evaluated parameters compared to control. Whole genome bisulfite sequencing analysis revealed widespread hypermethylation, particularly in genes associated with energy metabolism, transcription regulation, and DNA repair pathways. Notably, differentially methylated regions were enriched in fertility-related gene clusters. Small RNA and long non-coding RNA analyses identified numerous differentially expressed small RNAs and novel long non-coding RNAs in heat-stressed sperm. Several downregulated small RNAs were associated with spermatogenic regulation and chromatin remodeling. Long non-coding RNAs were found to co-express with genes involved in oxidative stress response and cellular apoptosis. Immunohistochemical evaluation showed marked increases in nuclear 5-methyl cytosine staining and repressive histone modifications in spermatogenic cells of the 36°C group. PCNA staining was reduced, indicating decreased proliferative capacity, while histone modifications associated with gene activation were also diminished. Increased expression of both Hsp70 and Hsp60 was observed, supporting the presence of a strong cellular stress response. Histopathological analysis revealed degeneration and disorganization of seminiferous tubules, marked reduction in the germinal epithelium, and significant loss of germ cells. In addition, the presence of germ cells resembling oocytes was observed, which was interpreted as a clear indication of partial feminization. Flow cytometric analysis confirmed a substantial decline in sperm viability and mitochondrial function, along with elevated oxidative stress, enhanced lipid peroxidation, and increased rates of apoptosis in the 36°C group. Computer-assisted sperm analysis revealed the lowest motility scores in this group, with sharp reductions in velocity parameters such as straight-line and average path velocities, as well as an increased frequency of morphological abnormalities. Fertilization trials using sperm from heat-stressed males and oocytes from control females demonstrated a marked decrease in fertilization and hatching success in the 36°C group, providing strong evidence that thermal stress leads to epigenetic and cellular disruptions that impair reproductive performance.

**Conclusion**

This study demonstrates that chronic heat stress induces profound epigenetic and physiological changes in the testes and sperm of Nile tilapia. The observed changes include hypermethylation of key fertility-related genes, altered non-coding RNA expression, increased histone repressive marks, and clear histological damage. These molecular and cellular disruptions were strongly associated with decreased sperm function and reduced fertilization success. Collectively, these findings suggest that specific epigenetic markers could serve as predictive biomarkers for thermal stress-induced subfertility and may aid in the development of thermotolerant Nile tilapia lines for sustainable aquaculture.

# CONDITIONING OF RECLAIMED URBAN WASTEWATER FOR REUSE IN AQUAPONICS

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**Introduction**

Reclaimed water aquaculture for food production exists outside Europe, especially in resource-poor regions. However, currently there is no capacity for reclaimed water aquaculture or aquaponics in Europe mainly due to low public acceptance and lack of regulations [1, 2]. The EU Water Reuse Regulation of 2020/741 [2] regulates the use of reclaimed water for agriculture, laying the groundwork for an extension to aquaculture. The novelty in this work pioneers the use of reclaimed water to farm fish and vegetables fit for human consumption in an aquaponic system, leading not only to a notably water scarcity alleviation but also a reduction of produce prices and carbon footprint avoiding transport costs.

For the first time in Europe, an experimental aquaponic farm has been coupled to a nearby urban wastewater treatment plant in Castellana Grotte, Puglia, Italy within the frame of the project *Aquaponics from wastewater reclamation (AWARE)* GA N. 101084245. Advanced (post-tertiary) treatment of wastewater has been tailored to feed the aquaponic farm to produce lettuce and Tilapia. The performance of advanced post-tertiary treatment and the aquaponic farm has been evaluated not only in terms of chemical and microbiological water quality, but also fish welfare, plant health and food safety. Here, we report on water quality.

**Materials and Methods**

The advanced tertiary treatment has been developed by Aquasoil. MITO3X technology is composed of an ozonation step with the ability to perform ozonation at a dose of 4.5 mg/L $O_3$ with nearly zero contact time (< 10 seconds) excluding the formation of brominated byproducts (bromates), follow by a vacuum ultraviolet (VUV) with an emission at a wavelength of 185 nm. This radiation can directly photolyze water and produce hydroxyl radicals and hydrated electrons, both species able to effectively degrade micropollutants. Then, a reactive storage (biofilter) is used downstream the ozone/VUV system. The biofilter is comprised of two tanks with a total dimension of 15 $m^3$ (5 m x 3 m x 1 m). Water is first subjected to granular activated carbon (GAC) and then, expanded clay and lava rock. This adsorption step can assure total retention of micropollutants. Assuming an average flow rate of 1 $m^3$/h, this ensures an average empty bed contact time (EBCT) of 15 hours. The biofilter is aerated using oxygen wasted by the ozone generator.

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These systems comprise various compartments (in this case: fish tank, biofilter, sump, DWC floating raft crop cultivation system, settler, and aerobic digester, provided by Green in Blue). In this study two aquaponic lines were studied to ensure reproducibility. Each aquaponic system was set up with three tanks of 350 L each, filled up with reclaimed wastewater, and stocked with a total of 140±6 Nile tilapia (*Oreochromis niloticus* L., initially stocked at 37.5±0.95 g per system) and a 5 $m^2$ of crop planting surface with 36 lettuces. The experiment was conducted in a small scale, closed-loop, simple recirculating aquaculture system (RAS) running for three months. An aquaponic line with dechlorinated tap water was used as control.

Several physico-chemical and biological parameters were monitored not only after the water advanced (post-tertiary) treatment, but also throughout the cycle in the sump of the aquaponic system. In particular, microplastics; metals; organic emerging micropollutants; human enteroviruses such as Sapovirus (SaV), Norovirus (NoV GI, NoV GII), Hepatitis (HAV, HEV), crAssPhage, and Pepper Mild Mottle Virus (PMMoV) which is considered as potential indicator of fecal contamination in aquatic environments; parasites (18S rRNA gene metabarcoding); fecal contamination bacteria indicators such as enterococci, total coliforms, *Escherichia coli*; and antibiotic resistance biomarkers and the bacterial community (16S rRNA gene metabarcoding) were analysed.

**Results**

Regarding the efficacy of the advanced (post-tertiary) treatment for the removal of contaminants, 77±5 organic micropollutants were detected in the inlet of the advanced (post-tertiary) treatment using non targeted screening conducted by high-resolution mass spectroscopy. After the treatment, an overall removal of 51.1% was measured in terms of sum of micropollutants. Furthermore, the stage of the reactive storage was able to achieve an additional removal of micropollutants reaching (relative to the influent) nearly two logs reduction (i.e., 98.7%). Suspect PFAS were not detected in the effluent. Metals were detected in trace concentrations in the inlet and effluent. Microplastics were reduced in 66%. The crAssPhage and PMMoV concentration were quantified in the inlet corresponding to $8.57 \cdot 10^2$ and $2.55 \cdot 10^5$ gc/L respectively. Moreover, 30% of PMMoV particles showed infectivity. SaV, NoV GI, GII, HEV, HAV were not detected (<LoD) or detected below LoQ. After treatment crAssPhage was totally inactivated and PMMoV was reduced to 1-log but no infectivity was confirmed. Bacterial counts were also below LoD. Regarding microbial water quality evolution in the aquaponic system, no viruses were detected through time, not even PMMoV. Accumulation of bacterial contamination was observed through time in the sump, but no *E. coli* was observed. The water microbial community was observed to change during the experimental cycle, and mirrored that of the biofilter, but not of the of fish and plants being cultivated. Various parasitic species were identified, but none have pathogenic significance. Interestingly, no differences in water quality were detected within the aquaponic system using dechlorinated tap water and reclaimed urban wastewater.

**Conclusions**

Advanced post-tertiary treatment was demonstrated to provide high quality reclaimed water. The combination of oxidation processes coupled to a final adsorption led to high reduction of microbial pathogens, micropollutants and microplastic particles. Its reuse in aquaponics showed promising results in comparison with potable water systems.

**Literature**

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# LIFE CYCLE ASSESSMENT OF RECLAIMED WASTEWATER-FED AQUAPONIC FARM

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## Introduction

Aquaponics is an unconventional method of fish and crop production, which combines aquaculture with hydroponics. Aquaponics can be installed in rocky, saline and no-arable lands, with no requirements for fertilisers and insecticides/herbicides. This contributes to reducing soil occupation and deterioration, alleviating the pressure on water resources, and reducing agriculture impact on the environment [1].

Urban wastewater is increasingly used for agriculture due to increasing water scarcity and stress, together with expanding populations, rising wastewater disposal levels and contamination episodes. Urban ecosystems should consider developing new markets for wastewater, diverting a higher percentage of wastewater inland, making productive use of wastewaters not only for agriculture but also for aquaculture and aquaponics [2,3]. For example, reclaimed water can reduce the geographical dependency of aquaculture to freshwater, so that it can be located anywhere near a city with wastewater treatment plants. This would result in a decrease in production costs and the procurement of fresh produce of high protein content to the benefit of local communities [4]. However, low public acceptance and lack of regulations are the main barriers [5].

The main goal of this work is to prove the reduction of environmental footprint of using reclaimed water in aquaponic farms assuring productivity and food safety within the frame of the project *Aquaponics from wastewater reclamation (AWARE)* GA N. 101084245. To do this an experimental small-scale aquaponic farm has been coupled to a pilot-scale urban wastewater treatment plant at URJC, Madrid, Spain. Advanced tertiary treatment of wastewater has been tailored to feed the aquaponic farm to produce lettuce and Tilapia. Environmental performance of reclaimed urban wastewater-fed aquaponic farm has been evaluated.

## Materials and Methods

Water advanced tertiary treatment was used to polish wastewater effluent from a wastewater treatment plant (WWTP). This process was composed of serial modules of Ultrafiltration (UF), electrooxidation (EO) and radiation with a total of 60 UVC-LED. The UF membrane is $Al_2O_3$, size pore of 100 nm, 0.23 $m^2$ x 1.2 m. The anode of the EO module corresponds to Ti/MMO (mixed metal oxides) and Ti is used as cathode, 270 $cm^2$, 5.2 A, 10-15 V, 78.85 J. The wavelength of UVC-LED radiation is in the range of 268-280 nm, with maximum emission of 275 nm, and energy of 102.99 J. The flow rate corresponded to 200 L/h.

In this study 1 $m^3$ reclaimed urban wastewater-fed aquaponic system stocked with 50 Nile tilapia (*Oreochromis niloticus L.*, stocked at 5.7 kg per system) and 72 lettuce plants (*Lactuca sativa* Batavian Red) for 22 lettuce cycles of 5 weeks. The experiment was conducted in a small scale, closed-loop, simple recirculating aquaculture system (RAS) running for 19 weeks. Control with dechlorinated tap water was also performed as base line.

A gate-to-gate life cycle assessment (LCA) was used following a standardised methodology by ISO 14040 and 14044. The selected functional unit (FU) was 1 kg of Tilapia. Mass allocation was applied to distribution environmental impacts and burdens across co-products. To prepare the inventory data the ecoinvent v3.8 was used, and the cut-off options for the unit process were selected. The reference method proposed by the European Commission, Environmental Footprint (EF) 3.0, was employed. To quantify the potential environmental impacts SimaPro 9.4 was used.

## Results

The main system features (including raw materials for building and running the facility); consumptions (energy and water); and production (fish feed, fish, lettuce, dead biomass, sludge) are shown in Table 1. The advanced tertiary treatment was also accounted for raw materials and energy.

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Table 1. Life Cycle Inventory (LCI) data of dechlorinated tap water-fed aquaponic system.

| Inventory Data-Dechlorinated Tap water (19 Weeks) | Quantity | Units |
|---|---|---|
| **Outputs from technosfere. Products and co-products** | | |
| Tilapia (mass allocation 4%) | 1.9 | kg |
| Lettuce (mass allocation 96%) | 45.1 | kg |
| **Inputs from technosfere (materials/fuel)** | | |
| Ammonium chloride {GLO}\| market for \| Cut-off, U | 0.014 | kg |
| Tilapia feed, 24-28% protein {GLO}\| market for tilapia feed, 24-28% protein \| Cut-off, U | 0.83 | kg |
| Polystyrene foam slab {GLO}\| market for \| Cut-off, U | 1.47 | kg |
| Tap water {Europe without Switzerland}\| tap water production, conventional treatment \| Cut-off, U | 3570 | kg |
| Expanded clay {GLO}\| market for \| Cut-off, U | 540 | kg |
| EDTA, ethylenediaminetetraacetic acid {RER}\| market for \| Cut-off, U | 0.55 | kg |
| Activated carbon, granular {GLO}\| market for activated carbon, granular \| Cut-off, U | 0.44 | kg |
| Polyethylene, high density, granulate {Europe without Switzerland} \| Cut-off, U | 82.5 | kg |
| Extrusion, plastic pipes {RER}\| production \| Cut-off, U | 82.5 | kg |
| Steel electrogalvanized steel/EU | 106 | kg |
| Glass fibre {RER}\| production \| Cut-off, U | 100 | kg |
| **Inputs from technosfere (Electricity/Heat)** | | |
| Electricity, medium voltage {ES}\| market for \| Cut-off, U | 3142 | kWh |
| Electricity, medium voltage {ES}\| market for \| Cut-off, U | 112.1 | kWh |
| Water pump operation, electric {ES}\| water pump operation, electric \| Cut-off, U | 335.2 | kWh |
| Electricity, medium voltage {ES}\| market for \| Cut-off, U | 670.3 | kWh |
| **Outputs to the environment** | | |
| **To water** | | |
| Nitrate | 61.7 | g |
| Nitrite | 0.22 | g |
| Phosphate | 3.26 | g |
| Ammonium, ion | 0.27 | g |
| TOC, Total Organic Carbon | 43.6 | g |

## Conclusions

Electricity use (pumps, lights, heaters) is the main contributor to Global Warming Potential together with energy demand depending on climate, and lighting. Nutrients in effluent discharge affect eutrophication potential. Comparing aquaponics using dechlorinated tap water with one using reclaimed urban wastewater introduces key environmental trade-offs. The reclaimed water reduces freshwater demand and embodied energy, but may introduce higher treatment energy.

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# EURCAW-AQUA: THE EUROPEAN UNION RESEARCH CENTER FOR ANIMAL WELFARE FOR AQUATIC ANIMALS

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## Introduction

On January 2024 the European Commission designated European Union Reference Centre for the welfare of aquatic animals (EURCAW-Aqua) (Commission Implementing Decision 2024/266). The centre was launched in September 2024. This EURCAW has similar objectives and mandates of the three previous existing EURCAWs focused on the welfare of pigs; ruminants and equines; and poultry and small farmed animals. Reference Centres aim at improving the enforcement of the legislation on animal welfare. The lead of EURCAW-Aqua was awarded to a consortium composed by the University of Crete (Greece), the Biology Centre of the Czech Academy of Sciences (Czech Republic), and the Universitat Autonoma de Barcelona (Spain).

## Objectives and mandate

The objective of EURCAW-Aqua is to provide services to National Competent Authorities (NCAs) in Member States, addressing the main scope to improve the welfare of aquatic animals and the enforcement of the current legislation on animal welfare.

The EURCAW-Aqua will establish a portfolio of competitive services for the European Commission and National Competent Authorities (NCA) of EU member states, based on sound scientific knowledge and operational excellence.

The designation of the EURCAW-Aqua is a very important step to fulfil the gaps in scientific and technical knowledge regarding aquatic animals. EURCAW-Aqua is currently developing validated animal welfare indicators, welfare assessment methods and tools for the improvement of the welfare status of farmed aquatic animals (fish, cephalopods and decapods) in the EU. The EURCAW-Aqua is also providing technical support and advisory services, coordinating assistance to NCAs in carrying out official controls, and disseminating good practices, operating as an information and resources hub. EURCAW-Aqua provides the NCAs with access to case studies, research and scientific findings, technical data, novel welfare tools, techniques and technologies to improve on farm aquatic animals' welfare assessment and the enforcement of the current animal welfare legislation and provides scientific evidence. The EURCAW-Aqua furthermore supports horizontal activities of the Commission and of the MS in the area of welfare requirements for aquatic animals. All relevant material will be publicly available through the Centre's web page (https://www.eurcaw-aqua.eu/). EURCAW-Aqua provides workshops, training courses and training material both to civil servants, inspectors and officers working in EU institutions and NCAs of member states on a free of fee basis.

To cover the need for firsthand official information about the activities of the EURCAW-Aqua and to support the Centre with technical information, a Reflection Board (REB) was set up. The REB represents the farmed aquatic animals supply chain, relevant professional organisations, civil society organisations, and relative advisory councils and reference bodies of EU interest. The European Commission is invited as an observer. The REB meets annually and the exact composition of the REB is defined during in the first year of operation of EURCAW-Aqua.

To address ethical considerations in organizational conflict, an Ethics Committee was established. The role of the Ethics Committee is the establishment of ethical guidelines and a code of conduct for the staff and partners of the EURCAW-Aqua.

## Structure

The EURCAW-Aqua is led by the University of Crete and has the following structure: a coordination team, an Executive Committee and an Operational Team. The coordination Team has three representatives of each Consortium Institutions. The Executive Committee includes the Coordination team members plus the Scientific coordinator and the Project Manager. The Operational Team includes experts in communication and organization devoted to the development of scientific and technical studies, the organization of training courses and the compilation of welfare indicators information.

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**Activities planned and performed so far**

The activities of the centre involve the following areas: (1) Support and coordinated assistance to national and regional authorities, (2) Animal welfare indicators, methods for the assessment and methods of improvement, (3) Conducting thescientific and technical studies, 4) Organizing training activities, and (5) Disseminating research findings and innovation activities.

During the 2024 Work Programme the Centre produced the following studies: (1) a regulatory framework review regarding live farmed farmed aquatic animals transport and harvest, (2) a list of relevant welfare indicators for common carp (*Cyprinus carpio)*, and European sea bass (*Dicentrarhus labrax*) during transport and at harvest, (3) an overall on-farm score for the evaluation of common carp welfare at transport and harvest, (4) a report on currently available welfare assessment systems for farmed fish, and (5) a blueprint for research to detect loss of consciousness and/or sensibility of fish at slaughter. These outputs are available on EURCAW Aqua portal. Three workshops have been organized during 2024 on specific subjects of interest for the NCAs. The first EURCAW-Aqua online workshop on rainbow trout welfare, was devoted to exploring welfare indicators and assessment methods for farmed rainbow trout. Attendants of NCA, particularly State Veterinary Inspectors, gained insights from leading experts in fish welfare, genetics, health, and behaviour. The second workshop was specifically designed for NCAs to provide targeted knowledge and practical tools for assessing and enhancing welfare in carp aquaculture, with a special focus on harvest, transport and slaughtering practices. The objective of the third workshop was to inform about the state of the art of the current advances and knowledge gaps on European seabass welfare, particularly in stunning and slaughter methods. Advances were reviewed in connection with the results of research projects (PerformFISH, MedAID, Cure4Aqua) as well from guidelines of institutional organisations (EFSA) and producer organisations that were contextualised in the current legislation framework. The Work Programme for the next programming period (2025-27) has been submitted, evaluated and approved by EU authorities.

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# DISEASE BURDEN IN FISH FARMING: ASSESSING THE COSTS AND WIDER IMPACTS OF DISEASES

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**Introduction**

In many instances, the likelihood of disease occurrence and its associated impact has been traditionally perceived as an inevitable phenomenon—subject to randomness—that must be accepted as an intrinsic aspect of human life. A comparable mindset is often observed in sectors involving the cultivation and production of living organisms, such as agriculture, livestock, and aquaculture. It is well established that disease can exert a profound influence on these production systems; nevertheless, comprehensive analysis—particularly from economic and wider perspectives—has been relatively limited. In response to this gap, the concept of Disease Burden has gained significant traction in human medicine over recent years, emerging as a critical tool for evidence-based decision-making in both public and private healthcare systems. More recently, this paradigm has been increasingly adopted in the context of animal production, including aquaculture, where it provides a structured framework for quantifying the various impacts of disease and informing management strategies.

**Materials and methods**

The concept of Disease Burden, when applied specifically to fish farming, is currently being examined from two distinct yet complementary perspectives under the framework of EUPAH&W and the Horizon Europe Cure4Aqua project.

More specifically, within EUPAH&W, this concept is being addressed under Priority Area 5 – Integrated Approach Including Socio-Economic Aspects of Animal Health and Welfare, and more concretely through Specific Objective Action 22 (SOA 22). This action assesses the economic and societal burden and impacts of selected priority diseases and production diseases, including diseases of terrestrial and aquatic farmed animals, on animal welfare, public health, and the environment. This action aims to support better decision-making by evaluating the costs and benefits of disease prevention, enhancing the sustainability of the EU livestock industry through improved health and welfare practices. It also develops bio-economic simulation models.

Concurrently, within the Cure4Aqua project, one of the tasks under Work Package 2 (WP2) focuses specifically on estimating the Disease Burden. This effort aims to quantify the impact of disease in aquaculture systems, providing a data-driven basis for prioritizing interventions and guiding policy and management strategies across the sector.

A preliminary analysis of how the concept of disease burden has been developed within the field of aquaculture—and more specifically in fish farming—revealed that, although certain studies and publications have addressed specific components of the concept in a targeted and applied manner, these efforts have been fragmented and lacked comprehensive coverage of all relevant dimensions. As a result, a more in-depth analysis was deemed necessary to establish an integrative and holistic understanding of the disease burden in this sector.

**Results and discussion**

The outcome of this analysis, conducted by the research team within the Cure4Aqua project during 2023 and 2024, has led to the development of a comprehensive review on the subject (Padrós et al., 2025). This work primarily identifies and elaborates on the following key aspects related to the impacts of disease in fish farming:

-The impacts of mortality and morbidity on the economy and performance of the farm.

-Economic evaluation of the costs associated with the management of diseases: surveillance, diagnostics, treatment, prevention, and biosecurity investments.

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-Evaluation of costs and benefits related to disease diagnosis and control.
-Evaluation of cost (also environmental) and benefits of therapeutics, non-medicinal, and other disease control strategies.
-Cost of the investments related to risk assessment and prevention at the farm level, including immunoprophylaxis and vaccination
-Impact of disease on farmed fish welfare
-Disease risk assessment, insurance, and quality assessment
-Societal impacts and governance implications
-Impacts of fish diseases and public health: a one health approach

These elements are currently utilized in the development of a novel economic analysis model (Glucksman et al., 2023) that is both flexible and adaptable, capable of supporting disease-specific assessments—such as sea lice infections in Atlantic salmon or sparicotylosis in gilthead seabream—as well as integrative analyses at the farm or enterprise level.

Furthermore, this review will serve as a foundational reference for analyses within Specific Objective Area 22 (SOA 22), as well as within the broader context of the entire Partnership for the years to come.

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# IMPACT OF DRYING METHOD ON THE SENSORY EVALUATION OF SHRIMP CHIPS USING THE JAR SCALE

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## Introduction

The growing demand for healthy and functional snacks, driven by consumers seeking convenient and health-promoting options, has led to integrating seafood and aquaculture products into snack development. These products respond to current consumer preferences for options high in quality protein, low in fat, and rich in micronutrients such as vitamins and minerals (Javier Pisco et al., 2024; Carlucci et al., 2015). However, for these products to gain market acceptance, it is crucial to address challenges related to stability, flavour, texture, and nutritional value, with particular emphasis on achieving positive sensory acceptance. This study aimed to evaluate the sensory characteristics of shrimp-based "chip" snacks subjected to two dehydration processes (conventional hot-air drying and freeze-drying), using the Just-About-Right (JAR) scale.

## Materials and methods

The raw materials used in this study included farmed shrimp (*Penaeus vannamei = Litopenaeus vannamei*) (12–16 g) provided by the Aquaculture and Biodiversity Research Group (GAB) of the Institute of Animal Science and Technology at the Universitat Politècnica de València. Additional ingredients—rice flour, potato starch, mashed potato, and salt—were purchased from a local supermarket, along with 28 mm Ø Nojax® cellulose casings (Viskase®, Lombard, Illinois, USA). The processing of the chips followed these steps: cleaning, heading and peeling of the shrimp; mincing; marinade under refrigeration (4±1°C for 20 minutes); stuffing into cellulose casings; cooking (80±2°C for 20 min); cooling; freezing at –18±2°C for 24–48 hours; slicing into 3.5 mm thick slices; and dehydration using either conventional hot-air drying (HAD) (90 minutes at 100±5°C) or freeze-drying (FD)( -50 ºC y 0.05 mbar for 72 h). Sensory perception was assessed using the Just-About-Right (JAR) test. The variables analysed included colour, characteristic seafood aroma, seafood flavour, texture (crispiness) and perceived saltiness. A five-point structured scale was used to determine the degree of appropriateness of each attribute regarding consumer preferences. An incomplete block design was applied, and the tasting panel consisted of 28 untrained participants, following a balanced experimental design.

Table 1. Percentage of consumers who have scored the descriptors using the jar method.

| drying treatment | Attributes | score (% consumers) | | |
|---|---|---|---|---|
| | | Excessive | Just About Right | Too Little |
| HAD | colour | 48 | 38 | 14 |
| | Seafood Aroma | 38 | 43 | 19 |
| | Seafood Flavour | 52 | 33 | 14 |
| | Saltiness | 67 | 29 | 5 |
| | Crispiness | 33 | 43 | 24 |
| FD | colour | 0 | 9 | 91 |
| | Seafood Aroma | 45 | 27 | 27 |
| | Seafood Flavour | 50 | 36 | 14 |
| | Saltiness | 32 | 59 | 9 |
| | Crispiness | 9 | 23 | 68 |

HAD: hot air drying; FD: freeze drying

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**Results**

The table 1 show the value of JAR results of the attributes evaluated. In the case of the hot-air dried (HAD) samples, significant deviations from the ideal point were observed across several attributes. Saltiness was the most affected, with approximately 67% of consumers perceiving it as too intense, negatively impacting the overall acceptance score. This excessive saltiness may lead to consumer rejection, as it is a critical attribute in snack-type products. Similarly, attributes such as colour, seafood aroma, and seafood flavour were perceived as too strong by many panellists (over 40% in some cases), contributing to a lower overall product perception. Crispiness, on the other hand, showed a more neutral behaviour, with minor deviations.

In contrast, the freeze-dried (FD) samples exhibited a more balanced sensory profile. Most attributes were rated as "just about right," except colour, which was perceived as "too little" by 91% of participants. However, some consumers rated the saltiness and seafood flavour as "too much," their impact on the mean acceptance score was less pronounced than in the HAD samples. Only crispiness was perceived as excessive by a small portion of the panel, without significantly affecting overall acceptance.

**Conclusions**

Overall, the data suggest that freeze-drying promotes a more stable sensory profile by reducing extremes in consumer perception. In contrast, hot-air drying intensifies specific attributes excessively, particularly saltiness.

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# IMPACT OF DRYING METHOD ON THE SENSORY EVALUATION OF SHRIMP CHIPS USING THE JAR SCALE

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## Introduction

The growing demand for healthy and functional snacks, driven by consumers seeking convenient and health-promoting options, has led to integrating seafood and aquaculture products into snack development. These products respond to current consumer preferences for options high in quality protein, low in fat, and rich in micronutrients such as vitamins and minerals (Javier Pisco et al., 2024; Carlucci et al., 2015). However, for these products to gain market acceptance, it is crucial to address challenges related to stability, flavour, texture, and nutritional value, with particular emphasis on achieving positive sensory acceptance. This study aimed to evaluate the sensory characteristics of shrimp-based "chip" snacks subjected to two dehydration processes (conventional hot-air drying and freeze-drying), using the Just-About-Right (JAR) scale.

## Materials and methods

The raw materials used in this study included farmed shrimp (*Penaeus vannamei = Litopenaeus vannamei*) (12–16 g) provided by the Aquaculture and Biodiversity Research Group (GAB) of the Institute of Animal Science and Technology at the Universitat Politècnica de València. Additional ingredients—rice flour, potato starch, mashed potato, and salt—were purchased from a local supermarket, along with 28 mm Ø Nojax® cellulose casings (Viskase®, Lombard, Illinois, USA). The processing of the chips followed these steps: cleaning, heading and peeling of the shrimp; mincing; marinade under refrigeration (4±1°C for 20 minutes); stuffing into cellulose casings; cooking (80±2°C for 20 min); cooling; freezing at –18±2°C for 24–48 hours; slicing into 3.5 mm thick slices; and dehydration using either conventional hot-air drying (HAD) (90 minutes at 100±5°C) or freeze-drying (FD)( -50 ºC y 0.05 mbar for 72 h). Sensory perception was assessed using the Just-About-Right (JAR) test. The variables analysed included colour, characteristic seafood aroma, seafood flavour, texture (crispiness) and perceived saltiness. A five-point structured scale was used to determine the degree of appropriateness of each attribute regarding consumer preferences. An incomplete block design was applied, and the tasting panel consisted of 28 untrained participants, following a balanced experimental design.

Table 1. Percentage of consumers who have scored the descriptors using the jar method.

| drying treatment | Attributes | score (% consumers) | | |
|---|---|---|---|---|
| | | Excessive | Just About Right | Too Little |
| HAD | colour | 48 | 38 | 14 |
| | Seafood Aroma | 38 | 43 | 19 |
| | Seafood Flavour | 52 | 33 | 14 |
| | Saltiness | 67 | 29 | 5 |
| | Crispiness | 33 | 43 | 24 |
| FD | colour | 0 | 9 | 91 |
| | Seafood Aroma | 45 | 27 | 27 |
| | Seafood Flavour | 50 | 36 | 14 |
| | Saltiness | 32 | 59 | 9 |
| | Crispiness | 9 | 23 | 68 |

HAD: hot air drying; FD: freeze drying

*(Continued on next page)*

**Results**

The table 1 show the value of JAR results of the attributes evaluated. In the case of the hot-air dried (HAD) samples, significant deviations from the ideal point were observed across several attributes. Saltiness was the most affected, with approximately 67% of consumers perceiving it as too intense, negatively impacting the overall acceptance score. This excessive saltiness may lead to consumer rejection, as it is a critical attribute in snack-type products. Similarly, attributes such as colour, seafood aroma, and seafood flavour were perceived as too strong by many panellists (over 40% in some cases), contributing to a lower overall product perception. Crispiness, on the other hand, showed a more neutral behaviour, with minor deviations.

In contrast, the freeze-dried (FD) samples exhibited a more balanced sensory profile. Most attributes were rated as "just about right," except colour, which was perceived as "too little" by 91% of participants. However, some consumers rated the saltiness and seafood flavour as "too much," their impact on the mean acceptance score was less pronounced than in the HAD samples. Only crispiness was perceived as excessive by a small portion of the panel, without significantly affecting overall acceptance.

**Conclusions**

Overall, the data suggest that freeze-drying promotes a more stable sensory profile by reducing extremes in consumer perception. In contrast, hot-air drying intensifies specific attributes excessively, particularly saltiness.

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# PARASITIC PRESSURE AND SEASONAL MORTALITY FLUCTUATIONS IN FARMED GILTHEAD SEA BREAM (*Sparus aurata*) CAUSED BY *Sparicotyle chrysophrii*

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**Introduction**

Fish diseases represent a major challenge for aquaculture, affecting fish health, welfare and growth, ultimately reducing productivity and increasing costs. The monogenean gill parasite *Sparicotyle chrysophrii* is a significant threat to gilthead seabream farming in the Mediterranean (Mladineo et al., 2023; Antonelli et al., 2010). This study examined the seasonal occurrence and mortality associated with *S. chrysophrii* infections in seabream reared in sea cages in the Gulf of Corinth under real farming conditions over the course of one year. The findings underscore the critical need for the implementation of seasonal parasite monitoring and targeted management interventions to effectively mitigate the detrimental impacts of *Sparicotyle chrysophrii* on gilthead sea bream (*Sparus aurata*) aquaculture.

**Materials and methods**

Mortalities and their causes were recorded for each sea cage used in the farming of gilthead seabream and entered into the electronic production management system of Galaxidi Marine Farm in Greece throughout the year. A statistical analysis was conducted to assess the seasonal occurrence of *S. chrysophrii* and the associated mortality due to parasitic infection. In addition, key production parameters were recorded, including average fish weight, stocking density, the month of introduction to sea cages, water temperature and farm unit. Group comparisons were performed using non-parametric Kruskal-Wallis tests followed by appropriate post hoc analyses. Furthermore, two machine learning techniques were applied in R to investigate the relationship between observed mortality and farming characteristics: Random Forest (via the ranger package) and Support Vector Machine (SVM) (via the e1071 package). The dataset included a wide range of numerical and categorical variables, with mortality rate as the primary response variable of interest.

**Results and conclusions**

The highest mortality rates attributed to *S. chrysophrii* infections in gilthead seabream were recorded during the summer and autumn months, with peak values observed in July and October **(Figure 1)**. This trend suggests that the temperature profile during these seasons favors the parasite's biological cycle, resulting in continuous reinfection of the farmed fish. Moreover, a correlation was observed between parasite-induced mortality and fish that were stocked in sea cages during the autumn and winter months. Another notable finding was the increased mortality in cages containing fish of higher average body weight, potentially linked to the absence of effective therapeutic interventions against the parasite. Stocking density also emerged as a significant factor influencing mortality rates, where cages with the highest stocking densities exhibited the highest parasite-related mortality **(Figure 2)**. Ultimately, the value of the collected data lies in its potential to support the development of more robust epidemiological models, improve predictions related to climate change impacts on parasitic infections, and guide the design of more structured and targeted sampling strategies. These insights offer promising opportunities for future research directions in Mediterranean aquaculture.

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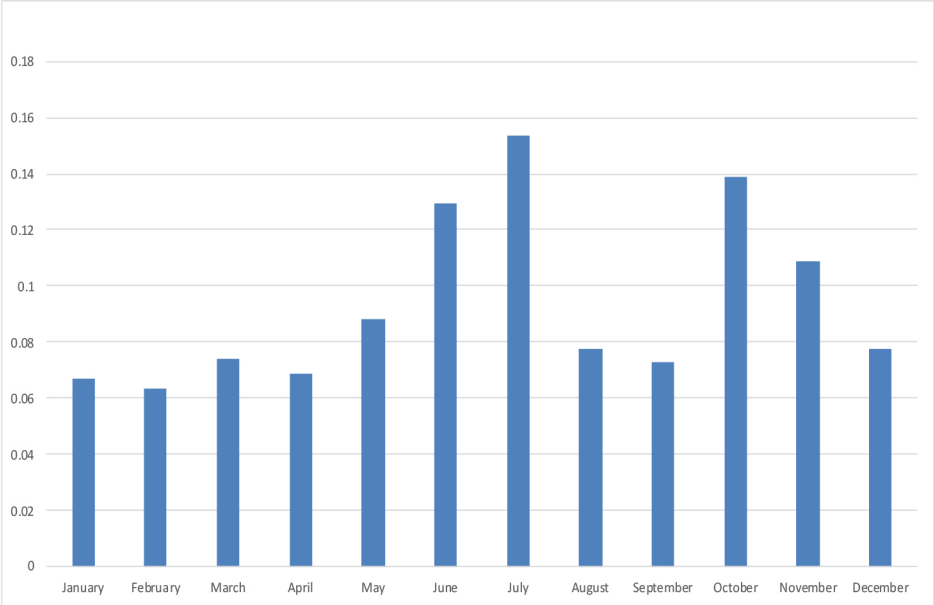


**Figure 1.** Monthly percentage mortality rates due to *Sparicotyle chrysophrii*

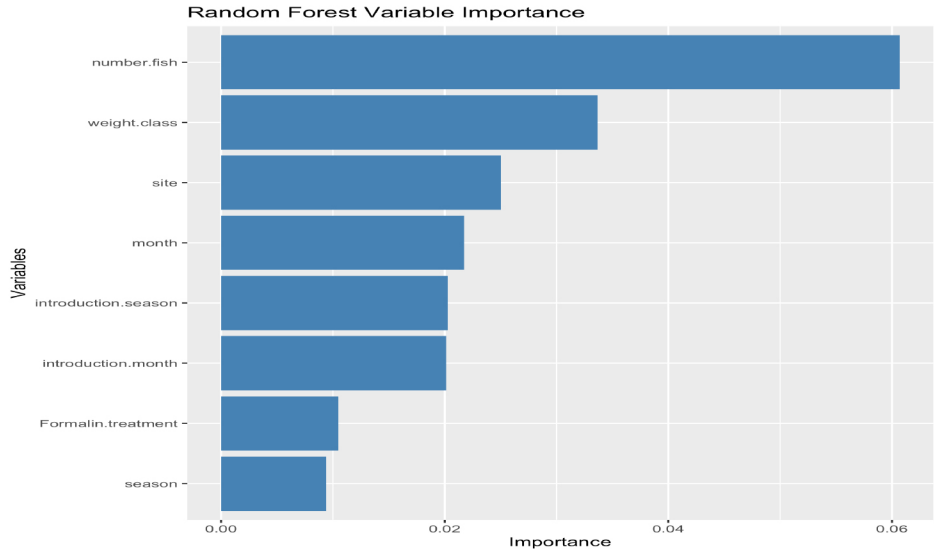


**Figure 2.** Correlation of Various Production Parameters with Mortality After Random Forest Analysis. The size of the bar indicates the importance of each parameter in the observed mortality

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# *Aeromonas veronii* IN EUROPEAN SEABASS (*Dicentrarchus labrax*): CLINICAL, MOLECULAR AND EPIDEMIOLOGICAL INSIGHTS FROM THE GREEK AQUACULTURE

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## Introduction

*Aeromonas veronii* has emerged as a significant bacterial pathogen in European seabass (*Dicentrarchus labrax*) aquaculture across the Aegean Sea (Smyrli et al., 2019, 2017). While traditionally overshadowed by other agents such as *Vibrio anguillarum* and *Photobacterium damselae* subsp. *pisicida*, *A. veronii* has increasingly been linked to systemic infections, severe mortalities, and economic losses in intensive marine fish farming. The disease affects primarily commercial-sized fish but has also been reported in juveniles under 50 g. Initially restricted to specific regions within the Aegean Sea (East Greece), recent reports indicate a concerning geographic spread to previously unaffected areas, including the Ionian Sea (West Greece). This trend is particularly alarming given the recent relocation of a significant portion of seabass production to these regions in an effort to escape earlier outbreaks.

In this study, we present new epidemiological data on *A. veronii* infections in Greece, detailing clinical signs, disease progression, and associated pathology. Furthermore, we evaluate current management strategies and treatment options, with particular emphasis on the role of autogenous vaccines as a key preventive measure.

## Materials and methods

Extensive sampling of European seabass exhibiting clinical signs of disease was carried out from April 2024 to April 2025 in the Igoumenitsa Bay region of western Greece. Fish of various size classes were clinically evaluated at both the Irida Fish Disease Diagnosis Laboratory and the Hellenic Centre for Marine Research (HCMR) in Crete.

Bacterial isolation was performed using standard microbiological techniques. Kidney tissues were streaked onto a range of nutrient media, including Tryptic Soy Agar (TSA), Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar, and Blood Agar. Isolates were characterized phenotypically (e.g., pigment production, motility) and biochemically using API 20E and Biolog GEN III systems.

Initial molecular identification involved sequencing of the 16S rRNA and *gyrB* genes. Selected *A. veronii* strains underwent whole genome sequencing (WGS) using DNBSeq and Illumina platforms to assess genomic similarity with previously identified strains, as well as to investigate virulence-associated genes and outer membrane protein (OMP) profiles.

## Results

The first signs of disease appeared in late April, when water temperatures were relatively low (16–17 °C). Initial mortalities were low but persistent. Clinically, affected fish exhibited lethargy, anorexia, ulcerative skin lesions, exophthalmia, and a distinctly icteric appearance. Internally, splenomegaly was observed, with white to cream-colored nodules of varying sizes present in the spleen, as well as nodules and abscesses in the kidney (Figure 1).

Mortalities increased progressively with rising water temperatures, peaking in late August at 28–29 °C. All bacterial isolates were identified as *Aeromonas veronii* biovar *sobria*. The newly isolated strains from Igoumenitsa were motile and showed variability in pigment production. Whole genome phylogenetic analysis revealed that these strains are more closely related to isolates from the eastern Aegean Sea (near the Turkish coast) and are genetically distinct from those previously isolated from the western Aegean (near the Peloponnese). Genomic analysis has shown that the bacterium possesses a very broad toolbox of virulence factors. Several antigenic surface proteins are conserved across different strains, however distinct differences exist between strains according to the geographic area of isolation.

Antibiotic treatment was administered based on antimicrobial susceptibility testing. Most isolates were sensitive to the antibiotics currently approved for use in aquaculture. However, disease management often required multiple treatment cycles. Stress was found to be a critical exacerbating factor, and careful feeding management—especially the avoidance of overfeeding—was essential during periods of elevated water temperature.

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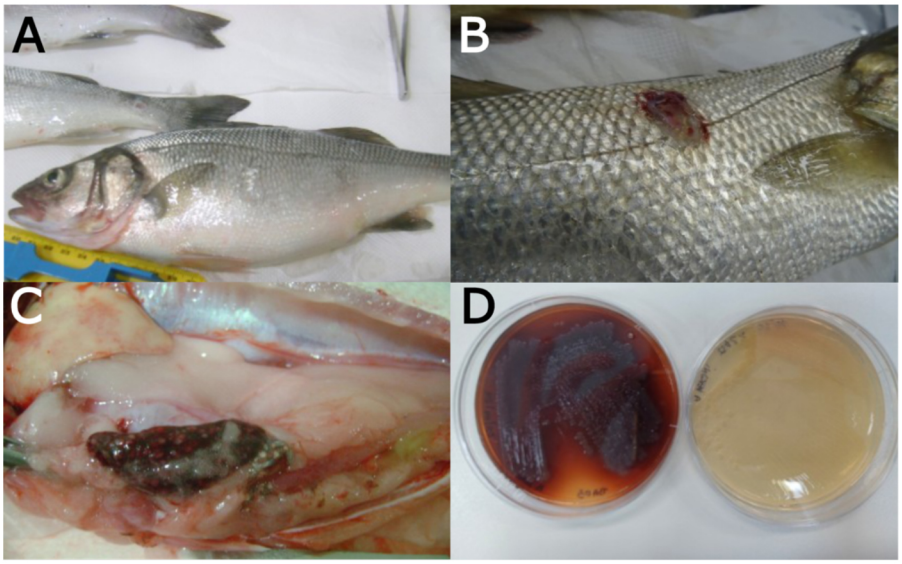


**Figure 1. A.** European seabass exhibiting exophthalmos, icteric skin, and abdominal distension suggestive of ascites. **B.** Focal ulcerative skin lesion. **C.** Splenomegaly with the presence of multiple splenic nodular lesions. **D.** Colony morphology of *Aeromonas veronii* on Blood Agar and Mueller-Hinton medium (evident hemolysis and pigment production, respectively).

## Discussion

*Aeromonas veronii* is now recognized as one of the most significant bacterial pathogens impacting European seabass aquaculture in the eastern Mediterranean, particularly in Greece and Turkey, where the majority of production is concentrated (Smyrli et al., 2019). Although this pathogen has been associated with disease outbreaks in a wide range of cultured fish species worldwide (Triga et al., 2022), including freshwater and marine species, it has not been detected in gilthead seabream (*Sparus aurata*), despite co-cultivation in the same facilities. This host specificity is notable and warrants further investigation.

At present, disease control relies primarily on antibiotic therapy—a short-term and unsustainable solution, especially since outbreaks often occur in fish nearing market size. While no commercial vaccines are currently available, the application of autogenous vaccines has shown encouraging results in reducing mortality and disease severity (Smyrli et al., 2022). Advances in molecular and genomic characterization of regional *A. veronii* strains are laying the groundwork for the development of targeted vaccines and more refined disease management approaches.

Ongoing monitoring and functional studies are essential to deepen our understanding of the pathogen's transmission routes, resistance potential, and seasonal patterns of occurrence, ultimately contributing to more sustainable and species-specific aquaculture health strategies.

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# IMPACT OF SALINITY ON THE MODULATION OF ENERGY METABOLISM IN CLAMS *Ruditapes decussatus, Venerupis corrugata* AND *Ruditapes philippinarum*

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**Introduction**
Salinity is a critical factor for the survival and development of clams in the Galician estuaries, where heavy rainfall and reservoir discharges cause fluctuations that dilute seawater. In the context of climate change and water resource management, innovative approaches are urgently needed to protect these ecosystems and sustain their economic activity (Kersting *et al*., 2016). Salinity affects clam metabolism, altering energy reserves such as glucose, glycogen, triglycerides, and fatty acids. While oxygen consumption and energy expenditure changes have been documented (Peteiro *et al*., 2018), alterations in key metabolites remain poorly understood. This study addresses this gap by analyzing metabolites from glucose, fatty acid, and amino acid pathways in commercially valuable clam species exposed to varying salinities (35‰, 12‰, and 6‰) over different exposure times (0, 2, and 4 days).

**Materials and methods**
Specimens of *Ruditapes decussatus*, *Venerupis corrugata*, and *Ruditapes philippinarum* were acclimated at 35‰ salinity under controlled temperature and oxygen conditions, then exposed to salinities of 35‰ (control), 12‰, and 6‰ for 2 and 4 days to mimic environmental variability. At each time point, soft tissues were harvested and homogenized in PCA buffer (0.8 N). Key energy metabolites (glucose, glycogen, lactate, triglycerides, and fatty acids) were quantified via spectrophotometry using commercial kits. Statistical analysis employed two-factor ANOVA with post-hoc tests to determine significant differences between treatments.

**Results**
Glucose levels decreased significantly in *R. decussatus* and *V. corrugata* across all treatments. Glycogen showed a downward trend without statistical significance. Lactate remained stable at 48 hours but decreased significantly at 96 hours, notably at 12‰ salinity. Triglycerides rose significantly at 24 hours in all species. Fatty acids decreased at 24 hours under 6‰ and 12‰ treatments and showed a pronounced, significant decline at 96 hours in *R. decussatus* and *V. corrugata*, whereas *R. philippinarum* displayed no significant changes.

**Discussion**
Our findings demonstrate that environmental salinity distinctly modulates metabolic parameters in *R. decussatus*, *V. corrugata*, and *R. philippinarum*. The marked glucose reduction in *R. decussatus* and *V. corrugata* reflects rapid energy mobilization under osmotic stress, consistent with accelerated substrate utilization under adverse conditions (Zhou *et al*., 2022). This metabolic response is linked to reproductive and other vital functions, as evidenced by Domínguez *et al*., 2021. The continuous yet nonsignificant glycogen decline indicates sustained consumption with limited replenishment capacity. Lactate stability in the short term followed by reduction at 4 days suggests adaptive metabolic adjustments that reduce lactate accumulation during prolonged stress (Peteiro *et al*., 2018). Significant increases in triglycerides suggest species- and context-dependent lipid storage or energy mobilization. Differential fatty acid declines between species indicate varied lipid mobilization strategies tied to salinity tolerance (Zhou *et al*., 2022). These results emphasize salinity as a crucial regulator of bivalve energy metabolism, revealing greater metabolic sensitivity in *R. decussatus* and *V. corrugata* compared to *R. philippinarum*, highlighting the need for tailored management approaches.

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**Conclusion**

Salinity reduction elicits species-specific energy metabolism responses in *R. decussatus*, *V. corrugata*, and *R. philippinarum*, reflecting distinct adaptation and stress mechanisms dependent on species and exposure duration. These results establish salinity as a key environmental factor modulating metabolic homeostasis and influencing survival and biological performance in Galician estuaries. The generated insights are vital for developing sustainable management and conservation strategies for natural and cultured clam populations under increasing environmental variability driven by climate change and anthropogenic impacts.

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# ENHANCING BREEDING DECISIONS WITH COST-EFFECTIVE METHYLOME SCREENING: THE CASE STUDY OF MALE FERTILITY IN ARCTIC CHARR (*Salvelinus alpinus*)

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## Information

The epigenome is an unexplored resource in aquaculture breeding. Among the known epigenetic mechanisms, DNA methylation is the most well-studied, and it is suggested that it underpins several key traits in fish (Piferrer, 2023). Despite the substantial drop in sequencing costs over the last decade, screening the methylome in sufficient numbers as required by selective breeding practices remains challenging. By utilizing reduced representation sequencing methods, we can obtain genomic and methylome information in a cost-effective manner. As a proof of concept, we used reduced representation sequencing to screen the sperm methylome of Arctic charr (*Salvelinus alpinus*) males and investigated potential links to fertility as previously suggested (Pappas et al., 2025)male fertility in fish remains understudied despite its critical importance to overall reproductive success in nature and captivity. This study investigated the DNA methylation landscape in spermatozoa of domesticated Arctic charr (Salvelinus alpinus.

## Materials and Methods

Fertility traits were recorded from 176 Arctic charr males from the Swedish breeding program. Sperm motility and kinematic parameters were measured using a Computer Assisted Sperm Analysis (CASA) system equipped with the SCA® Motility imaging software. Moreover, sperm concentration was counted using a NucleoCounter® SP-100™. Reduced representation sequencing libraries were prepared using methylation sensitive restriction enzymes. The library preparation followed a modified version of the original ddRAD protocol (Peterson et al., 2012) with previously documented adjustments (Franchini et al., 2017; Palaiokostas et al., 2015) , allowing at the same time scanning the methylome (Marconi et al., 2019). Sequencing was performed in one 10B lane of NovaSeq X Plus. Following sequencing and detection of single nucleotide polymorphisms (SNPs) using the Stacks software (Rochette et al., 2019), association studies for the recorded fertility traits were performed. Simultaneously, methylation levels were calculated and screened for putative associations with the male fertility traits using a weighted region comethylation network analysis with the R package comethyl (Mordaunt et al., 2022).

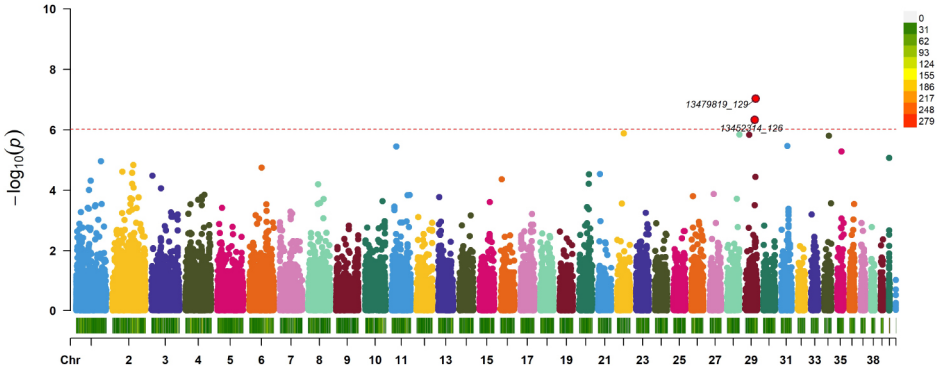


**Figure 1**. Association analysis for total motility in Arctic charr broodfish from the Swedish breeding program.

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**Results**

Approximately 15,500 SNPs satisfying quality control (found in at least 80% of the animals; minor allele frequency above 0.05; maximum heterozygosity of 0.6 and with a minimum minor allele count of 3) were retained for the association studies. Two SNPs located at chromosome 29 were found statistically significant at a genome-wide level (Figure 1; $p < 0.05$). In addition, 13 comethylated regions were constructed out of which one was found to have statistically significant association with sperm concentration ($p < 0.05$).

**Discussion - conclusions**

Using a cost-effective reduced representation protocol, we obtained genome- and methylome-wide information from over 170 Arctic charr males using one sequencing lane on an Illumina NovaSeq X Plus. As sequencing output continues to increase, our approach is expected to become even more cost-effective. Finally, we obtained novel information about the genome and methylome architecture controlling male fertility in Arctic charr.

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# SALICYLANILIDES INCLUDE HIGHLY EFFECTIVE CANDIDATE TREATMENTS AGAINST POLYOPISCOTYLEAN GILL PARASITES IN GILTHEAD SEABREAM (*Sparus aurata*)

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**Introduction**

The hematophagous ectoparasite *Sparicotyle chrysophrii* represents a critical health challenge for net-pen aquaculture of gilthead seabream, one of the main fish species cultured across the Mediterranean and the third most produced in Europe. Current treatment options are limited, with formalin baths being the only approved therapy in Western Europe. However, their application in sea cages is logistically complex, costly, and raises environmental, fish welfare, and operating safety concerns. This context highlights the urgent need for effective, safe, and practical in-feed antiparasitic alternatives tailored to the needs of marine aquaculture.

Our work focuses on investigating the mechanisms of action (MOA) of pharmacological active ingredients with potential against polyopisthocotylean infections. Stemming from previous computational virtual screening of chemical libraries for antiparasitic compounds and *in vitro* studies, several candidate drugs were shortlisted. In this work, we evaluate four drugs belonging to the salicylanilide chemical family: niclosamide, closantel, and their molecular fingerprint compounds ME1.62 and CG047.

**Materials and methods**

Adult specimens of *S. chrysophrii* were obtained from the gills of experimentally infected gilthead seabream. An *in vitro* dose-response assay was performed to evaluate the survival of parasites exposed to selected pharmacological compounds. The trials were conducted in duplicate, in 24-well plates, with 10 worms per well. Worms were observed at various times post-exposure (p.e.) using a binocular microscope, and survival was registered for each of the compounds. Survival data were analyzed using nonlinear regression models fitted to normalized dose-response curves in order to determine the lethal dose 50 (LD50), which is the concentration that results in a 50% mortality rate among the worms. We determined the LD50 at different times p.e., with special focus on the short-term effect (less than 2 hours p.e.).

Moribund or dead worms were fixed for histological examination to assess sublethal effects. We used scanning electron microscopy (SEM) to evaluate the effects of the treatments on the ultrastructure of the parasites' tegument upon exposure to the LD50_2h (n = 10 specimens/treatment). We also used immunolabelling approaches to investigate the MOA of the drugs at the level of the musculature and cytoskeleton.

**Results**

Three of the 4 salicylanilides tested resulted highly effective killing the parasites *in vitro*. According to their LD50 at 2h p.e, they ranked as follows (Figure 1): niclosamide (0.06792 $\mu$M), ME1.62 (0.08778 $\mu$M), closantel (2.294 $\mu$M) and CG047 (188.6 $\mu$M).

The primary MOA of this chemical family is related to uncoupling of the mitochondrial oxidative phosphorylation and eventually disturbing ATP production. Based on this, morphological analysis of exposed worms revealed an antero-posterior body contraction for the three most effective salicylanilides tested. Examination by SEM evidenced that the severe body contraction affected the surface of the parasites' tegument, compared to control untreated worms. The tegument formed deep transverse folds and ridges in all parasites exposed to the treatments. The parasite's haptor, the organ for attachment to the gill filaments provided with two rows of clamps, was also severely affected by the drug treatments. Muscle contraction provoked clamp opening after treatment with closantel and ME1.62, whereas niclosamide induced clamp opening accompanied by the retraction of the clamps within their tegumental sheaths. Additionally, it caused erosive damage in some tegument areas (Figure 2).

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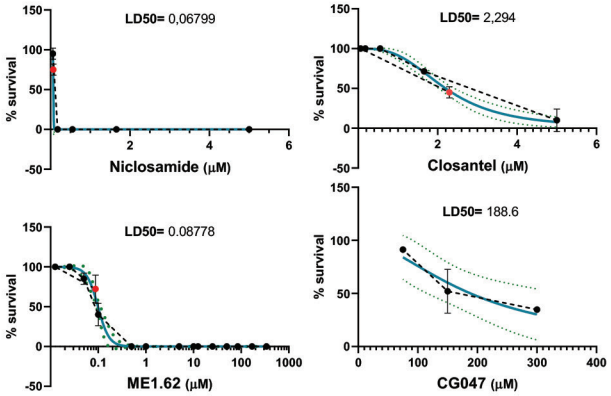


Figure 1. Lethal dose 50% (LD50) for *Sparicotyle chrysophrii* adults after 2h of salicylanilide treatments *in vitro*.

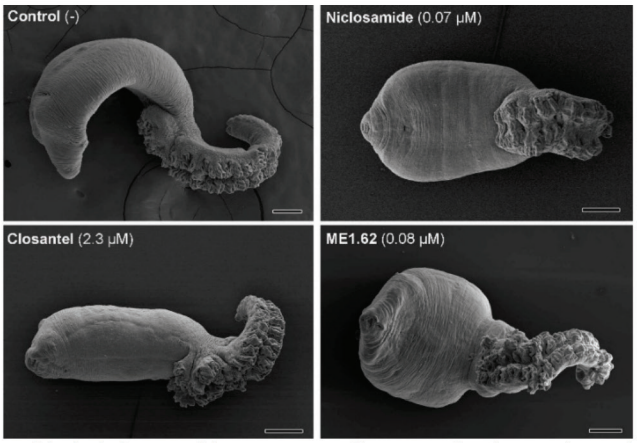


Figure 2. Effect of lethal dose 50% (LD50) on *Sparicotyle chrysophrii* adults after 2h of salicylanilide treatments (scale bars = 100 µm).

## Conclusions

Niclosamide and closantel are routinely used as antihelminthic agents in both human and veterinary medicine, targeting parasite motility and essential physiological processes. This study offers new insights into the mode of action (MOA) of three salicylanilide-based compounds, contributing to a deeper understanding of their efficacy and specific effects on *S. chrysophrii*. The primary MOA of this pharmacological class is linked to disruption of the parasite's energy metabolism, which in this study led to severe muscle fiber contraction, resulting in body shortening and clamp opening, ultimately followed by death. The high effectiveness of some tested compounds was particularly noteworthy, with niclosamide and ME1.62 demonstrating efficacy at doses more than 1,000 times lower than praziquantel, an anthelmintic with known effectivity against polyopisthocotyleans.

## Funding

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# THE EFFECT OF PHOTOPERIOD ON SWIM BLADDER INFLATION IN EURASIAN PERCH LARVAE – A CHALLENGING STEP TOWARD UNDERSTANDING EARLY DEVELOPMENT

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## Introduction

Swim bladder inflation effectiveness (SBIE) is a crucial milestone in the early development of all fish species. While Eurasian perch (*Perca fluviatilis*) can survive without an inflated swim bladder, unsuccessful inflation is linked to reduced body size, skeletal deformities, increased energy expenditure from constant swimming, elevated cannibalism, and higher mortality (Czesny et al. 2005). From an aquaculture perspective, maximizing SBIE is therefore essential. Previous studies on percid larvae have shown improved SBIE in Eurasian perch reared in black tanks versus white (Palińska-Żarska et al. 2019), disruption of inflation by even low nocturnal light in yellow perch (*P. flavescens*) (Suchocki et al. 2019), and optimal inflation in pikeperch (*Sander lucioperca*) under a 16L:8D photoperiod (Panana et al. 2023). Despite these insights, no standardized photoperiod protocol exists for Eurasian perch. Although continuous light (24L:0D) promotes growth, it often results in suboptimal SBIE. This study tested three photoperiod regimes (8L:16D, 14L:10D, 24L:0D) to identify optimal light conditions for maximizing swim bladder inflation in Eurasian perch larvae.

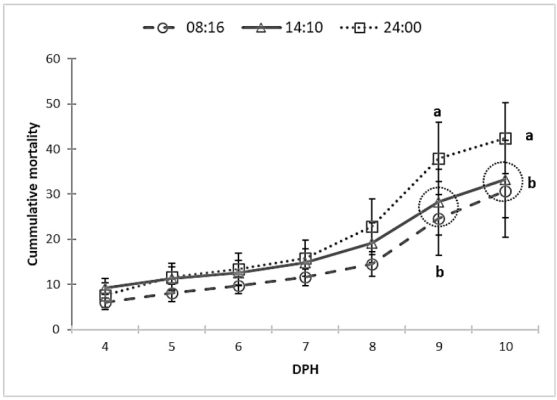


Fig. 2. Cummulative mortality during swim bladder inflation period in Eurasian perch larvae.

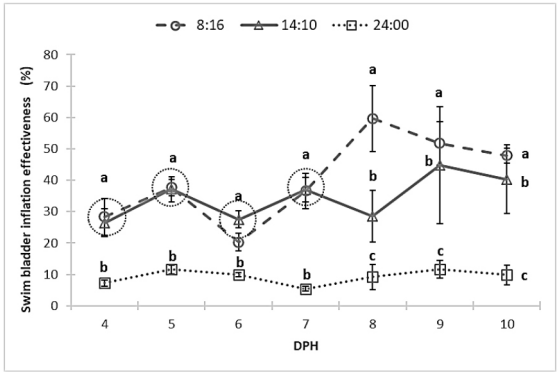


Fig. 1. Swim bladder inflation effectiveness of Eurasian perch larvae reared in three different photoperiods.

## Materials and methods

Eurasian perch embryos and larvae were reared under three photoperiods: 8 Light:16 Dark (8L:16D - minimum required for feeding and hygiene), 14L:10D (natural conditions during this time in nature), and 24L:0D (commonly used to enhance growth). After hatching, larvae were volumetrically counted and stocked at 3000 individuals per tank, with three replicates per treatment. From 4 days post hatch (DPH), larvae were fed *Artemia* nauplii *ad libitum* 3 times daily, following the protocol of Palińska-Żarska et al. (2020). SBIE (%) was assessed from 4 to 10 DPH by sampling ~100 larvae per tank and counting those with inflated swim bladders under a stereomicroscope. Mortality was recorded daily, and at 10 DPH, wet body weight (WBW, mg) and total length (L, mm) were measured.

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## Results

From 4 to 7 DPH, no significant differences in SBIE were observed between larvae reared under the 8:16 and 14:10 photoperiods ($p>0.05$). However, from 8 to 10 DPH, significantly higher swim bladder inflation rates were recorded in larvae kept under the 8:16 photoperiod compared to those reared under 14:10 ($p< 0.05$). Throughout the entire observation period (4–10 DPH), larvae exposed to continuous light (24:0) consistently showed the lowest SBIE values among all treatments ($p<0.05$) (Fig. 1). At 9 and 10 DPH, cumulative mortality was also significantly higher ($p<0.05$) in the 24L:0 group compared to the other two photoperiod regimes (Fig. 2). No significant differences ($p>0.05$) were found in WBW or total L of larvae at 10 DPH.

## Discussion

As a physoclistous species, Eurasian perch (*Perca fluviatilis*) larvae can inflate their swim bladders only within a limited developmental window . This brief and critical period poses a challenge in aquaculture, as suboptimal environmental conditions during this phase may lead to poor SBIE, compromising the performance and viability of the entire cohort. To date, no standardized larval rearing protocol exists for maximizing SBIE in percid species. In practice, hatcheries and scientists vary in their use of photoperiods, light intensities, and tank colors. For example, some studies have reported better SBIE in larvae reared in black tanks compared to white ones (Palińska-Żarska et al. 2019), although conflicting results have also been documented (Tamazouzt et al. 2000). In our experiment, the highest SBIE was observed under the longest dark-phase photoperiod (8:16), followed by 14:10. While inflation rates of 40–50% may not seem impressive at first glance, they are considered satisfactory for percid larval culture (Palińska-Żarska et al. 2019). Interestingly, the poorest SBIE and the highest cumulative mortality by 10 DPH occurred in larvae reared under continuous light (24:0). Although preliminary, our findings suggest that the presence of a dark phase („night") plays an important role in successful swim bladder inflation in Eurasian perch larvae. In light of the present findings, identifying the optimal photoperiod - and understanding, at both the molecular and physiological levels, how photoperiod influences the early developmental stages of Eurasian perch - emerges as a crucial direction for future research.

## Acknowledgment

This research was a part of the project "Exploration of the development, role and functioning of circadian rhythm in early life stages of *Perca fluviatilis"* funded by the National Science Centre, Poland (OPUS project, number UMO-2021/43/B/NZ9/03056).

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# WATER FLOW ENRICHMENT EFFECTS ON ACCELEROMETRIC ACTIVITY PATTERNS, GROWTH, BLOOD PARAMETERS, FILLET QUALITY AND WHITE MUSCLE RNASEQ OF YELLOWTAIL KINGFISH *Seriola lalandi* IN A COMMERCIAL MARINE RAS ENVIRONMENT

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**Introduction**

One of the few enrichment opportunities in commercial aquaculture facilities to boost fish growth, health and welfare is water flow enhancement. Particularly fast-growing athletic species with high metabolic demands for oxygen such as yellowtail kingfish grasp the benefits of flow enrichment and the associated swimming exercise (Palstra et al., 2015, 2024). This study aimed at establishing the physiological effects of water flow enrichment on yellowtail kingfish in commercial marine RAS production tanks.

**Materials and methods**

In the enriched flow tank (regular dodecagon with a volume of 62 $m^3$), flow was generated by a propellor and speeds ranged between 70-75 $cm.s^{-1}$ in the outer curve down to averages of 18-29 $cm.s^{-1}$ near an inner placed ring (Fig. 1). As a result, fish (N= 400 per tank as part of a total of 4,000 fish per tank; initial body-weight ~160 g) could swim at their preferred swimming speed where the maximum was optimal for fish at the start of the trial. The flow in the identical regular flow tank ranged from 13-17 $cm.s^{-1}$ down to averages of 6-10 $cm.s^{-1}$.

Overall Dynamic Body Acceleration (ODBA) was monitored with implanted acoustic transmitters with accelerometer sensors over the first three months (up to a BW of ~1,200 g) and used to demonstrate the increased activity of fish in the enriched flow tank vs. the regular flow tank (N=10 sentinel fish in each tank). Flow enrichment effects on growth, blood parameters, fillet quality and white muscle transcriptomics were determined after seven months (BW ~2,600 g).

**Results and discussion**

ODBA levels were 34% higher in the flow enriched production tanks (Fig. 1) where fish were most active at 7-8 h in the morning. The higher activity levels were supported by higher plasma glucose levels. Under enriched flow, less fish died (36% less than in the tank with regular flow), fish grew faster (10% during the first two months) and more homogenous, and fish had a leaner body shape (lower body height index). Fillet quality data are currently being analyzed while RNAseq data showed up-regulated keratin expression supposedly reflecting increased firmness.

Overall, we can conclude that flow enrichment is beneficial for growth and welfare of yellowtail kingfish in commercial marine RAS production tanks, thereby validating the earlier experimental-scale results (Palstra et al., 2015). If flow speeds could be further increased with increasing fish size, benefits may be more significant along the whole on-growing period. Acoustic sensortags with ODBA settings provide valuable information about the activity of the fish which could be extended to a digital twin model for energy expenditure and useful for precision farming.

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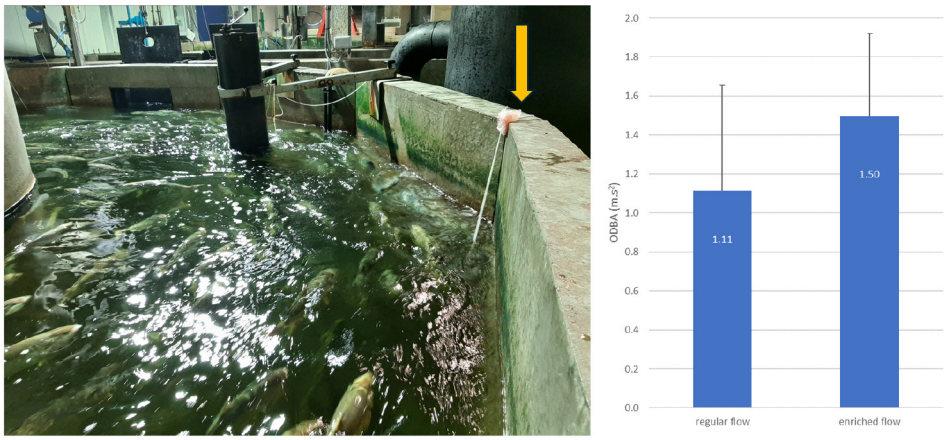


Figure 1. The picture illustrates the experimental fish swimming in the tank with inner ring under enriched flow conditions. The arrow indicates the position of the receiver for the sensortag signals. The graph shows the Overall Dynamic Body Acceleration (ODBA) values of experimental fish in the tank with regular flow and in the tank with enriched flow. Fish in the enriched flow tank showed an averaged value (± standard deviation) of $1.50 \pm 0.45$ m.s$^{-2}$ which was 34% higher than $1.12 \pm 0.54$ m.s$^{-2}$ for fish in the tank with regular flow ($P<0.001$).

**Acknowledgements**

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# A JOURNEY TOWARDS IDENTIFICATION OF PATERNAL-EFFECT GENES AND EXPLORATION OF THEIR ROLES DURING EARLY LIFE STAGES IN EURASIAN PERCH, *Perca fluviatilis*

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**Introduction**
The influence of paternal effects on progeny quality, particularly during early developmental stages, has historically been underappreciated. Recently, sperm selection procedures like cryopreservation have been started to be used to identify paternal-effect genes (PEGs; Panda et al., 2024). The expression of these genes in the progeny under the paternal control, meaning their expression rely on paternally-derived factors such as epigenetic status and/or non-genetic inheritance factors (e.g., ncRNAs) contained in the sperm cell. Sperm cryopreservation is a leading and sophisticated process for sperm preservation, keeping the sperm cell viable and functional for artificial reproduction (Cabrita et al., 2010). It is also been reported that sperm cryopreservation may affect the transcriptomic profile of the progeny (Wang et al., 2022). In Eurasian perch (*Perca fluviatilis*), comparative analysis of transcriptomic profile of freshly hatched larvae obtained following usage of fresh or cryopreserved sperm for fertilization allowed to identify a panel of novel PEGs (Panda et al., 2024). These findings show that exposing sperm to a specific challenge is an excellent approach to identify PEGs.

It is specific feature of cryopreserved E. perch sperm that after thawing its motility decreases over a relatively short period of time, with significant reduction after already 30 min. Thus, post-thaw storage seems to be additional challenging factor for the sperm cells keeping the most robust cells viable. Therefore, the aim of this study was to compare progeny obtained following using freshly thawed (0 min; CON) and stored after thawing (post-thaw stored sperm; PTS) for 30 min through their zootechnical performance and transcriptomic analyses. We hypothesized, that such additional selection pressure will enable to reveal new paternally controlled traits and PEGs.

**Methods**
The semen from wild males (n=6) were stripped, checked for their motility and concentration; and cryopreserved as described by Judycka et al. (2021). Fertilizations were done with eggs coming from each female (n=3) portioned equally. Cryopreserved sperm straws were thawed at 40°C for 10 seconds in a water bath, and poured into an Eppendorf, stored at 4°C for 30 minutes (PTS), while the same thawing procedure was used for 0 min, CON without providing them a waiting period and taken into use directly. A total of six pairs were created, with two males used to fertilize eggs from single female. Advanced larviculture was carried out as described by Palińska-Żarska et al. (2020) till 27 days post hatch (dph), while noting zootechnical parameters. Larvae at mouth opening (MO) stage, when larvae started weaning and at end (27 DPH) were sampled for transcriptomic and gene expression analysis.

Transcriptomic profile larvae at MO from both groups were compared using RStudio (version 4.1.3) using the package DESeq2 (Love et al., 2014). Differences were considered significant when corrected p-values were inferior to α (α=0.05).
Quantitative real-time PCRs (RT-qPCR) were performed to validate the chosen differentially expressed genes (DEGs), normalized using 5 housekeeping genes, to verify expression levels of those genes at MO stage; weaning stage and end stage if positively validated.

**Results**
Notably, larvae from the PTS group exhibited higher mortality rates compared to the CON group, with minor or none differences in terms of other zootechnical parameters. Transcriptomic analysis of larvae at the mouth-opening stage revealed 49 DEGs, many of which associated with immune-related functional pathways. Several genes, including *mfap*, *gimap*, *hlag*, *pigr*, *pde6g*, and *neo1*, emerged as promising candidates for PEGs.

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**Discussion**

High mortality in PTS group is a very strong phenotype and directed the study's focus towards paternal effects. This finding suggests that paternity may compromise immune functions to promote larval survival. Alongside, the gene *pde6g*, which plays a crucial role in phototransduction, can be confirmed to be a strong candidate as PEG, given its steep downregulation in the PTS group and occurrence in our previous studies identify PEGs.

Overall, this study challenges the long-held view that maternal effects predominantly govern larval development and phenotype. It also represents a significant step towards disentangling individual parental contributions, thereby advancing our understanding of the effect of reproductive techniques in aquaculture which may lead us toward fine-tuning the hatchery protocols. Besides, it broader our knowledge in the field of reproductive and developmental biology bringing us closer to understanding non-genetic inheritance in finfishes.

**Acknowledgements**

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# DOES BETA-GLUCAN FROM MUSHROOMS SOLVE SERIOUS BOTTLENECK IN COMMON CARP (*Cyprinus carpio*) PRODUCTION?

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**Introduction**

Production of common carp (*Cyprinus carpio*, hereafter referred to as carp) takes approximately 33 months and includes several stages performed in different types of ponds. The key challenges alongside the production process include carp translocations from one pond to another as fish grow, and the several-month (October-April) wintering period when water temperature drops significantly to 4°C and farmers cease feeding. Among the two wintering periods, the first one, which includes carp fingerlings, is the key bottleneck since fish mortality during this stage can reach up to 50% or more. One of the circular-based solutions that approach this issue of high mortalities is feeding carp fingerlings before wintering with beta-glucan retrieved from another agricultural sector, i.e. mushroom production. Oyster mushrooms (*Pleurotus ostreatus*) production in Europe is mainly localised in Spain, followed by Poland and Italy, and ends with the edible cap directed for human consumption and the by-product stem that is usually wasted or composted and re-enter the process of mushroom production as part of the medium. However, a high content of beta-glucan (up to 35%), a potent immune booster, can serve as an ingredient that can potentially influence the condition of carp fingerlings during wintering and improve survival rates. Therefore, the study aimed to improve survival rates and the condition of carp fingerlings during the wintering period by feeding them with feed enriched with beta-glucan from the mushroom stalls.

Material and methods

The fish trial included two main stages. First, for 8 weeks before wintering, 50 carp fingerlings with an average weight of 40 g were randomly distributed into 12 floating cages (triplicated variants) and fed four feeds enriched with 0% (control, var. A), 0.1% (var. B), 0.2% (var. C), and 0.4% (var. D) of beta-glucan powder from oyster mushroom stems. After that period, fish were translocated into wintering ponds for 6 months. Before the first and second stage, and after the wintering period, fish were sampled and a series of zootechnical parameters were assessed, e.g. total weight, Fulton's condition factor, viscerosomatic (VSI) and hepatosomatic (HSI) indices. Moreover, skin and proximal intestine samples were secured for histological analysis. Results were also compared to traditional practices at the carp farm (var. E), i.e. carp were cultured in fingerling ponds and transferred into the typical wintering ponds until sampled. The timing of the first and last sampling, and the transfer time for the wintering phase of fish in the cages, has been correlated with the typical farm practices for carp fingerlings.

**Results and discussion**

Compared to the traditional wintering of carp fingerlings (60% mortality rate), the survival rate of fish in the cages ranged from 100% to 82%, and the direct effect of the beta-glucan on this zootechnical parameter was not observed. The main findings for the other zootechnical parameters showed no significant loss of total weight of fingerlings after wintering, and an increase in the average weight of the fish from the traditional wintering, which might be attributed to more natural food resources accessible to carps freely swimming in the wintering pond. The other condition parameters (VSI, HIS) were significantly lower in fish from the wintering pond (var. E), and the lower values may indicate the lower condition of these fish, presumably due to the cumulative effect of low energy reserves before wintering and compromised nutrient availability during winter. The findings are also confirmed by the laboratory analyses, which mainly showed that the skin of the fish from cages was thicker and included more *dermis stratum compactum* that supports better adaptation for the wintering period. In the case of the intestine, histomorphological studies showed that fish fed with the highest level of beta-glucan (var. C and D) had the longest intestinal villi and the lowest number of Goblet cells. The morphological changes in the intestine may indicate that a higher inclusion of beta-glucan in the feeds leads to the development of the absorption area and more efficient uptake of nutrients. The study showed that feeding carp fry before the wintering period solves the severe bottleneck in common carp farming. However, further studies are needed to characterise the intrinsic mechanism behind the influence of beta-glucan on the common carp performance.

**Funding**

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# CIRCULAR AND INCLUSIVE UTILISATION OF ALTERNATIVE PROTEINS, BSF, *Hermetia illucens*, and Spirulina, *Arthrospira platensis* AS FISHMEAL REPLACEMENT IN DIET FOR EUROPEAN SEABASS, *Dicentrarchus labrax*

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**Introduction**

The aquaculture industry currently relies heavily on marine and land-based protein sources to meet the nutritional requirements of farmed fish and livestock. However, the rising cost of these raw materials, along with the environmental impact of their overexploitation, present a significant challenge to sustainable aquaculture production [1]. In response, there is growing interest in exploring more sustainable alternatives. These include plant by-products, macro- and microalgae, single-cell proteins, insect meal, and other unconventional sources [2]. As part of the CIPROMED project (PRIMA 2022,section 1), innovative feed ingredients such as insect meal (*Hermetia illucens*) and a blend of insect meal and microalgae (*Arthrospira platensis*) have been tested on European seabass (*Dicentrarchus labrax*) at the AquaBioTech testing facility in Malta to assess growth performance and somatic indices.

**Materials and Methods**

The trial was conducted in a 21 x 300L RAS system continuously monitored through an OxyGuard Pacific unit (DO, T, pH, ORP). A control diet and six experimental diets were formulated to be isonitrogenous, isolipidic and isoenergetic, replacing 100% of fishmeal with black soldier fly and spirulina meal (HI100 and SPI100 respectively), and a blend of both; Mix1 (80/20), Mix2 (60/40), Mix3 (40/60), Mix4 (20/80), and tested in triplicate. The diets were manufactured by Sparos Lda (Portugal).

European seabass, *Dicentrarchus labrax* (80 fish/tank; initial weight of 17.33 ± 0.05g), were randomly distributed at an initial stocking density of 4.6kg/$m^3$.

Fish were fed twice a day until satiation for 56 days. Bulk weighing of each replicate was performed at the end of the trial, and 3 fish from each tank were randomly selected and euthanized with an overdose of anesthetic to record biometrics such as total weight and length, liver and viscera weight. Growth performance (FCR, SGR, SFR%, feed intake) and somatic indexes (K, VSI, HIS), were evaluated and compared among diets.

**Results**

The results showed significant differences among the seven dietary treatments (CTRL, Hi100, MIX1, MIX2, MIX3, MIX4, and SPI100) across growth performance and somatic indexes.

Final body weight (FW) was highest in Mix1 (59.12 ± 1.33 g), followed by Hi100 (58.57 ± 0.68 g) and CTRL (57.36 ± 1.12 g), while significantly different to Mix3, Mix4 and SPI100, which exhibited the lowest FBW (46.31 ± 1.59 g). Feed conversion ratio (FCR) was significantly improved in Hi100 and Mix1 (1.05 ± 0.01, and 1.04 ± 0.02, respectively), while SPI100 had the poorest FCR (1.15 ± 0.06). Specific feeding rate (SFR) was highest in CTRL, Hi100, and Mix1 (ranging from 2.28–2.34%), and significantly lower in Mix3, Mix4 and SPI100 (ranging from 2.02–2.16%). Total feed intake showed significant variation, with CTRL, Hi100, and Mix1 consuming the most feed (3.40± 0.06 kg), while SPI100 consumed significantly less (2.41 ± 0.04 kg).

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Among the somatic indexes, condition factor (K), viscerosomatic index (VSI), and hepatosomatic index (HSI) did not exhibit consistent significant trends across treatments, though HSI was significantly higher in Hi100 (1.75 ± 0.25%) and Mix3 (1.79 ± 0.10%) compared to SPI100 (1.13 ± 0.27%).

The evaluation of both ingredients proved to be an efficient alternative to conventional fishmeal even at complete replacement.

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# NEXT-GENERATION FUNCTIONAL FEED IN AQUACULTURE: A PREBIOTIC STUDY WITH *Saccharomyces boulardii*

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**Objectives:**
Polyphenols derived from agri-food by-products are recognized for their diverse biological activities; however, their interaction with probiotic yeasts in synbiotic formulations remains insufficiently investigated. This study evaluates the effects of polyphenol-rich extracts from mulberry, olive, pomegranate leaves, and chestnut shells, on *Saccharomyces boulardii*, a probiotic yeast of emerging interest in aquaculture for promoting host health and reducing antibiotic dependency.

**Methods:**
Hydroethanolic extracts were obtained using a microwave-assisted extraction technique and subsequently characterized for their phytochemical profile and antioxidant capacity. The prebiotic potential of these extracts on *S. boulardii* was evaluated through a yeast fitness assay. At specific time points during incubation, both extracellular and intracellular postbiotic fractions were isolated. Extracellular postbiotics, recovered from the cell-free supernatants, were analyzed for polyphenol content, antioxidant activity, and soluble protein concentration. Intracellular constituents were extracted via cell lysis and subjected to the same analytical assessments.

**Results:**
All tested extracts exhibited significant total polyphenol content and antioxidant activity. Among them, olive and pomegranate leaf extracts notably enhanced the growth of *S. boulardii* in a glucose-free medium over a 7-day incubation, indicating strong prebiotic potential. Both extracellular and intracellular postbiotic fractions showed elevated levels of polyphenols, antioxidant activity, and soluble proteins compared to the control. Notably, the highest bioactivity in extracellular metabolites was observed with pomegranate leaf extracts, while intracellular fractions were particularly enriched following exposure to mulberry and olive leaf extracts.

**Conclusions:**
This study underscores the potential of polyphenol-rich agri-food wastes to promote *S. boulardii* growth and enhance postbiotic production under nutrient-limited conditions. These findings highlight their promising application in sustainable synbiotic formulations aimed at advancing eco-friendly aquaculture practices.

# A GAME FOR TWO: GENETIC PARAMETERS OF OBSERVED AND LATENT PARAMETERS OF FERTILITY IN SELECTIVELY BRED ARCTIC CHARR

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**Information**
Fertility is an important prerequisite directly affecting the success of selective breeding programs. Unlike livestock species, where unfavourable genetic correlations between production and reproduction have been reported, this relationship has been understudied in aquatic animals. In this study we investigate genetic parameters and correlations for a series of milt quality traits and growth in Arctic charr (Salvelinus alpinus) from the closed nucleus of the Swedish national breeding program. This population has been subject to selection for approximately 40 years (nine non-overlapping generations), with notable genetic gains for growth rate but also low and variable fertilization success rates (Palaiokostas et al., 2021) that make breeding practice challenging.

**Materials and Methods**
Using state-of-the-art, fluorescence-based cell counting and a standardized protocol for Computer Assisted Sperm Analysis (CASA) we recorded sperm concentration and kinematic parameters from 1,106 male charrs over two spawning seasons. Variance components estimation was performed in a multi-trait fashion with REML using either pedigree information alone or a combination of pedigree and genomic information from ~1/4 of the individuals using single step methodology. Our models adjusted for effects of year and water temperature at the day of sampling when applicable. A similar multivariate analysis was also applied to assess covariance components across two sex-limited traits, namely egg-count and sperm concentration, together with female and male growth proxies.

In addition, using retrospective data from 576 crosses we developed a statistical framework to disentangle latent fertility traits and their respective genetic parameters under two Bayesian hierarchical models. Unobserved male and female fertility factors were considered in non-linear functions describing the fraction of spawned eggs that were fertilized and reached the eyed stage. Animal models for the underlying factors were used as sub-models and genetic variances were partitioned utilizing additive genetic relationships.

**Results**
The phenotypic variation in the studied traits was extensive and could explain part of the variation in fertilization success rates. Heritability estimates for milt phenotypes appear relatively low to moderate spanning from ~0.1 to ~0.3. At the same time, fork length, a phenotype used as a proxy of growth rate, had a relatively moderate to high heritability of ~0.6 and revealed negative genetic covariance with male fertility proxies. More specifically, genetic correlations of -0.4 to -0.5 (depending on model) were yielded between sperm concentration and fork length while estimates around -0.2 were computed for between the latter and most sperm motility/velocity parameters. Lastly, fecundity, which is moderately heritable (h2 of ~0.4) showed positive genetic covariance with female and male growth but weak negative genetic correlation with sperm concentration.

Genetic variance posterior estimates corresponding to the female and male fertility variables converged at high levels explaining from around half and up to ~90% of the total variance (though characterized by relatively large standard errors).

**Discussion**
Our analysis suggests low to moderate heritability estimates for sperm quality traits. While this is in line with preliminary studies in the same populations (Kurta et al., 2022), here we extend by examining additional parameters to obtain insights on the genetic architecture of reproductive success in Swedish Arctic charr. Such parameters are egg count, latent variables for female and male fertility and the genetic covariance of growth rate with fertility. Opposing genetic correlation signs between fertility and growth for females and males reveal a likely complex genetic architecture governed by trade-offs and sex-specific genetic interactions with possible implications for the future of selective breeding practice in the closed nucleus.

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Latent fertility variables seem to have a strong heritable component, with estimates notably higher than the heritabilities for fertility proxies. That said, the respective estimated breeding values for male fertility were positively correlated with those for key sperm phenotypes. Despite a certain level of uncertainty due to limited data dimensions, this might reflect that fertility is a non-linear composite of underlying factors, with only a subset of which having been used as proxies in this study.

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# A NOVEL METHOD FOR STURGEON SEXING BASED ON REAL TIME PCR

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## Introduction

The sturgeon is known as a 'living fossil', estimated to have existed for more than 250 million years, and is known primarily for its eggs, which are used to produce caviar, although its meat is also eaten. Virtually all caviar marketed today can be considered to come from aquaculture (EUMOFA, 2021). There are 25 sturgeon species belonging to the family *Acipenseridae*, among which the best known and most appreciated species belong to the genus *Huso* and *Acipenser*, the latter including the largest number of species.

At present, cytogenetical studies have not revealed any presence of sex heteromorphic chromosomes in sturgeons (Havelka et al., 2011). Due to their lack of secondary sexual characteristics, sturgeon sexing cannot be directly distinguished by their appearance.

It is noteworthy that caviar results from the processing of sturgeon eggs, so therefore requires the rearing of female fish. Thus, exploitation of sturgeons is highly costly, as females take many years to reach reproductive maturity. In fact, the process of selecting females for caviar production can be done after an average of 8 years of rearing, depending on the species, through an ultrasound examination to differentiate between male and female sturgeons. During this period, it is necessary to breed both males and females, with the consequent production (EUMOFA, 2021).

In recent years, new sex identification methods of sturgeons are based on specific genes or molecular markers. For example, it has been reported the amplification by PCR of the specific molecular marker fragments of sturgeon, and the subsequently detection of amplified fragments by gel agarose electrophoresis, for sex identification (Kuhl et al., 2021; Ruan et al., 2021). However, this methodology cannot meet the needs of current gender identification of sturgeons for the lack of accuracy and reproducibility.

For this reason, the industry needs accurate and robust sexing systems for sturgeon at an early age to adapt the feeding and needs of the animals as early as possible to optimise the maturation of the females and the final production of caviar.

## Material and Methods

Reference tissue samples of 296 adult sturgeons (*Acipenser baerii*, *A. gueldenstaedtii*, *A. naccarii* and *A. naccarii x A. baerii* (ANB)), were taken and stored at -20 °C until use. DNA extraction is performed using Wizard® Genomic DNA Purification Kit. All adults were previously sexed by visualization of the gonads (ovaries or testes) by ultrasounds in the facilities of Caviar Riofrío (Granada, Spain).
The amplification and detection of a 65 bp sex specific fragment is carried out by real time PCR amplification. Amplification is performed in MicroAmp Optical 96-well reaction plate. Each reaction mixture contained 0.3 μM of each forward and reverse primer*, 2.5 μM of specific FAM labeled probe* and 1-10 ng of template DNA. Amplification reactions are carried out employing TaqMan™ Universal Master Mix (Applied Biosystems, Thermo Fisher Scientific, USA). The analysis was carried out using the fluorescent signal level during the initial cycles of PCR. This baseline is used to accurately determine crossing point (Cp). Every sample is analyzed by triplicates and the average Cp is calculated for each sample.

* Primer and probe sequences are patent pending

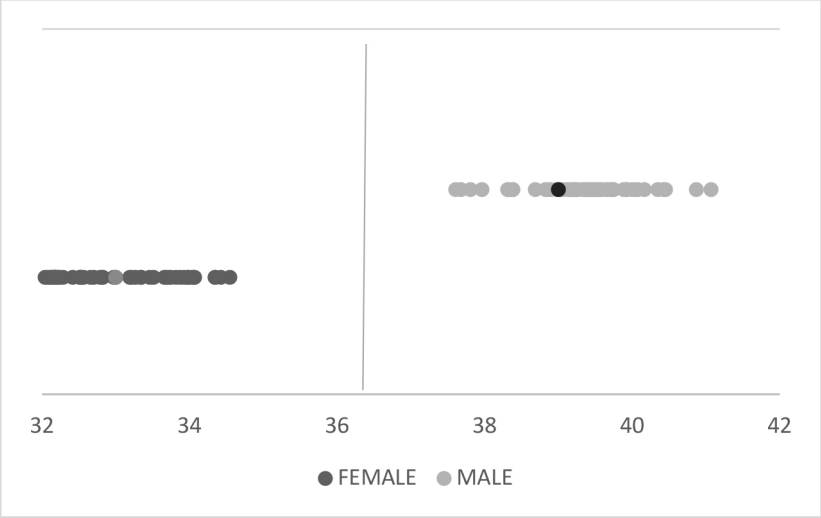


**Figure 1.** Validation of the sturgeon sexing method with the ANB species. The x-axis shows the Cp values. The vertical line represents the reference value of 36.52. Each point is the mean of the triplicates.

## Results and Conclusions

In the case of ANB species and after analyzing, at least, 50 male and 50 females, the real time PCR method has a 97,41 % correct male assignment (Figure 1). In other species, by far, the correct assignment is 100 % but, in the near future, a higher number of individuals are required to finish the complete validation with more sturgeon species.

In conclusion, the real time PCR method is suitable for sturgeon sexing of the most important commercial species. This molecular method has proven to be very accurate and reliable in comparison to other molecular methods previously described, holding the potential to become a routine methodology.

## Acknowledgments

Reference samples were kindly provided by Caviar Riofrio (Granada, Spain)

*Primer and probe sequences are patent pending

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# FLOW CYTOMETRIC CHARACTERIZATION OF HEMOCYTES OF THE SWORDTIP SQUID, *Uroteuthis edulis*

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The swordtip squid, *Uroteuthis edulis*, is a commercially significant marine resource in Jeju Island, Korea. However, little is known about its immune mechanisms, particularly cellular immunity. This study aimed to characterize hemocytes of *U. edulis* using flow cytometry to elucidate their immunological roles and potential application in resource management. Hemolymph samples were collected from the cephalic artery of squids acclimated for four days post-capture. Hemocytes were morphologically categorized using light microscopy and subsequently analyzed for cellular functions including total hemocyte count, phagocytic activity, reactive oxygen species (ROS) production, and lysosomal content. Flow cytometric analysis revealed three distinct hemocyte populations: large granulocytes, small granulocytes, and blast-like cells, with small granulocytes being the predominant type (approximately 80%). Functional assays demonstrated significant variations among these populations. Large granulocytes exhibited high phagocytic activity (30–40%), robust ROS generation ($3.3 \times 10^6$ arbitrary units), and elevated lysosomal content ($5.8 \times 10^5$ arbitrary units), suggesting their pivotal role in innate immunity. Conversely, small granulocytes showed minimal phagocytosis but moderate ROS production, while blast-like cells displayed negligible phagocytic activity and lower lysosomal content. These findings provide critical insights into the immunological profile of *U. edulis*, underscoring large granulocytes as key effectors in immune defense. This characterization provides essential baseline information to support fisheries resource management and serves as a fundamental indicator of responses to environmental stressors.

# GROWTH AND SURVIVAL OF JUVENILE MANILA CLAMS *Ruditapes philippinarum* IN TWO SUSPENDED SYSTEMS IN A SARDINIAN LAGOON (SOUTH ITALY)

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## Introduction

Bivalve mollusks play a vital role in coastal ecosystems and global aquaculture, contributing significantly to food security, nutrient cycling, and seafood-related economies. Among them, two clam species hold particular ecological and commercial importance: *Ruditapes decussatus* (the grooved carpet shell), native to the European Atlantic coasts and the Mediterranean, and *Ruditapes philippinarum* (the Manila clam), native to the Indo-Pacific region now naturalized in the Mediterranean Sea. Over recent decades, and increasingly in recent years, the clams' populations have undergone significant changes and are threatened by the growing frequency and intensity of extreme events (e.g., heatwaves and flash flood) in lagoon ecosystems coupled with the expansion of invasive species, such as the blue crab *Callinectes sapidus*. Indeed, a recent study reported that the blue crab shows a feeding preference for *R. decussatus* under mesocosm condition. According to FAO data, bivalves are among the most produced mollusks in aquaculture, with *R. philippinarum* being the second most cultivated bivalve species after oysters. Historically, Italy has been the leading European producer of clams and one of the top global producers, accounting for over 90% of European clam production in 2010. On the other hand, the species R. decussatus is traditionally harvested using artisanal fishery methods in the Mediterranean Sea, particularly in brackish coastal lagoons.

The objective of this study is to develop a pre-fattening system for clams that ensures an optimal cost-benefit ratio to strengthen clam supply chains. Specifically, we explored the effect of 1) different pre-fattening systems (lanterns and poches), 2) the effect of the presence of sandy substrate in lanterns (S vs B), 3) the effect of the installation depth in poches (SP vs BP). We considered clam growth and survival as response variables. The trials were tested in a suitable production area within the Santa Gilla lagoon, in collaboration with the fishery cooperative Consorzio Ittico Santa Gilla.

## Material and methods

For the trials we considered a homogeneous starting density of 0.2g/cm$^2$ of clams (0.13g/ind) in both lanterns and poches. The trials started in March 2024 (t0) and lasted four months. Bottom poches (BP) were placed at a depth of around 1.5m, while lanterns (L) and suspended poches (SP) were positioned within the top 20 cm of the water column. Each system was arranged along a long-line setup, spaced approximately 50 cm apart from each another.

Every month environmental parameters (temperature °C, Salinity and dissolved oxygen) were recorded using a multiparametric probe (*In-Situ* SmarTROLL Multiparameter Handheld). Additionally, a HOBO pendant data logger for temperature was placed *in situ*. At the same time, specimens were sampled and measured to estimate growth. Water samples were also collected to analyze the concentration of some inorganic nutrients (nitrate, nitrite, ammonia, total phosphorus). At the end of the trials all specimens were collected to estimate the survival rates under different conditions. Differences in clams' growth were assessed with univariate permutational analyses of variance (PERMANOVA), considering treatment and time as sources of variation.

## Results and Discussion

Biometric data (weight, length, width, thickness) of *R. philippinarum* were monitored over four periods (t0–t3) and showed significant differences in treatments and time, but not in their interaction. Weight increased progressively in all systems, with the highest gains observed in the Lantern Sand treatment. Systems using Poches, particularly the BP type, showed lower weight gain. Pairwise tests confirmed significant differences between t0 and t1 across all treatments, and among most system combinations at later time points. Length and width followed similar trends, with steady increases over time, particularly in

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lantern systems. Significant differences were detected across most group comparisons, except between some Poches treatments at later stages. Thickness also increased steadily, with the highest gains again seen in Lantern Sand. In summary, Lantern systems, especially those with sand substrate, consistently outperformed Poches systems across all biometric parameters. Moreover, survival was higher in the Lantern system (75–78%) compared to the Poches (47–52%).

This pilot study evaluated the effectiveness of different pre-fattening systems for clams in the Santa Gilla lagoon, comparing two cages: lantern and poches, with and without sandy substrate. Results showed that lantern systems with sand significantly improved clam growth and survival, supporting previous findings. However, higher stocking densities negatively impacted survival rates, highlighting the need for thinning practices and regular biofouling removal. Lantern systems were more reliable in offering protection and stability to the juveniles. The lagoon's nutrient-rich but eutrophic environment showed oxygen and nutrient levels suitable for clam farming, although continuous monitoring is essential, especially during periods of environmental stress. The observed growth rates aligned with previous studies from other Italian lagoons and confirmed the suitability of Santa Gilla for clam aquaculture. Nevertheless, improved management strategies are needed, such as increasing cleaning frequency and optimizing stocking densities. These findings also emphasize the need for adaptive practices to face challenges such as extreme weather events and variable water quality.

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We acknowledge financial support under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.5 - Call for tender No.3277 published on December 30, 2021 by the Italian Ministry of University and Research (MUR) funded by the European Union – NextGenerationEU. Project Code ECS0000038 – Project Title eINS Ecosystem of Innovation for Next Generation Sardinia – CUP F53C22000430001- Grant Assignment Decree No. 1056 adopted on June 23, 2022 by the Italian Ministry of Ministry of University and Research (MUR).

# LEVERAGING PUBLIC TRANSCRIPTOME DATA TO EXPLORE CELL TYPE AND TISSUE SPECIFIC EXPRESSION OF TARGET GENES IN SALMONID FISH

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## Introduction

Understanding the expression patterns of key regulatory genes is critical for elucidating their biological roles across different cell and tissue types. In fish species, such insights are especially important for advancing research in aquaculture, developmental biology, and environmental adaptation. Here, we leverage publicly available transcriptome datasets from the Gene Expression Omnibus (GEO) database to investigate both bulk and single-cell RNA sequencing (RNA-Seq) expression profiles of mucosa-associated lymphoid tissue lymphoma translocation protein (*malt*) gene orthologs in salmonid fish species, with a focus on rainbow trout (*Oncorhynchus mykiss*), a key salmonid representative. Utilizing a combination of bioinformatic pipelines, including transcript quantification, single-cell RNA-Seq methods, and differential expression analyses, we systematically mapped the expression landscapes of three malt paralogs present in rainbow trout across a range of tissues and cell types.

## Material and methods

The workflow followed in this study is as follows:

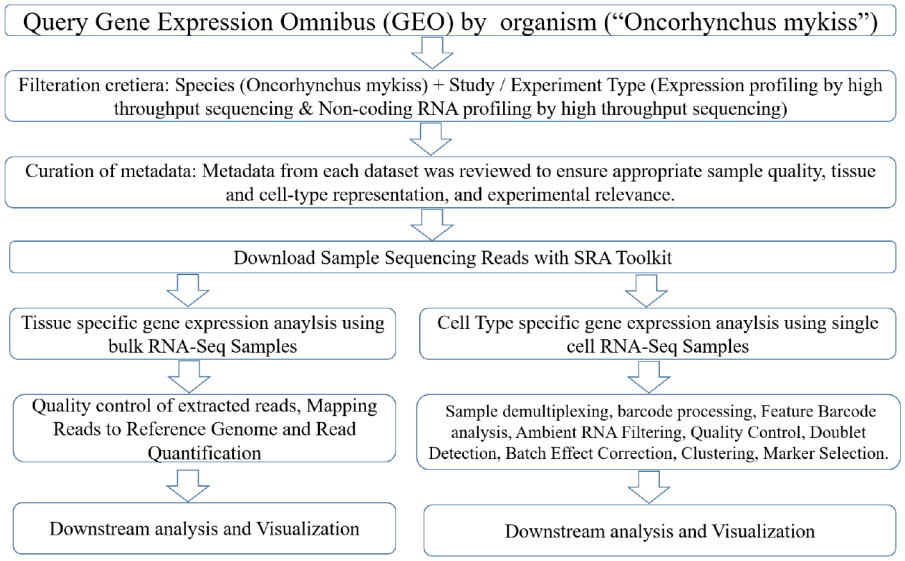


**Figure 1: Workflow for the meta-analysis of public datasets**

## Results and discussion

Initial quantitative RT-PCR validation revealed that *malt1* expression is predominant in the head kidney of rainbow trout, while *malt2* shows elevated levels in the spleen and trunk kidney. In contrast, *malt3* exhibits higher expression in the liver and intestine compared to other tissues. These findings highlight a functional diversification of MALT paralogs that may reflect specialized immune and metabolic roles across different tissue environments.

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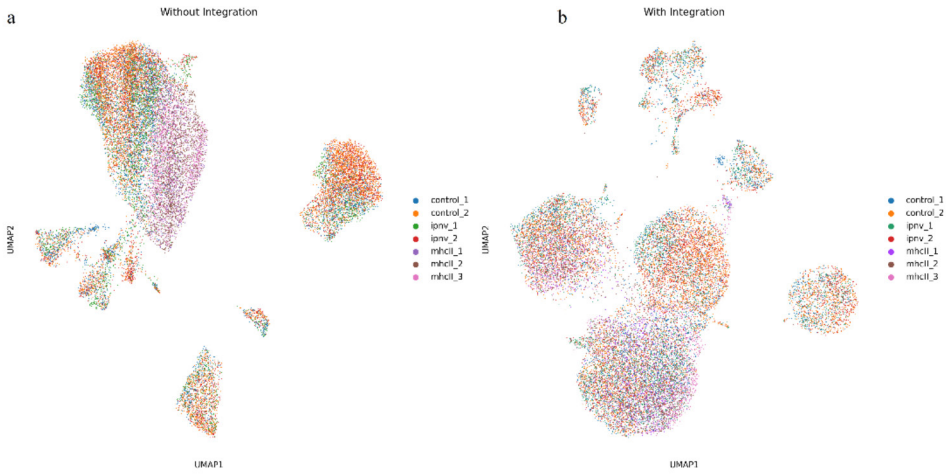


**Figure 2: Visualization of samples from merged GEO datasets (GSE260891 and GSE158102) (a) without integration and (b) with integration using scvi-tools.**

To further investigate these patterns, a meta-analysis combining bulk RNA-Seq and single-cell datasets is currently in progress. Integrating heterogeneous single-cell data, which encompasses diverse cell-type compositions, remains a considerable challenge. In this study, we demonstrate that the Single-cell Variational Inference (scvi) framework offers a more effective strategy for harmonizing diverse single-cell datasets (Lopez et. Al, 2018) (Figure 2).

Our results reveal distinct, tissue-specific expression patterns of the three *malt* paralogs in rainbow trout. By integrating public transcriptomic resources with targeted experimental validation, our study underscores both the opportunities and challenges of functional genomics in aquatic organisms.

Furthermore, these insights lay the foundation for a deeper exploration into the regulatory mechanisms of immune and stress-response pathways in salmonids, with potential applications in fish health management and aquaculture improvement.

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# THE EFFECT OF TEMPERATURE ON FOOD CONSUMPTION OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) CO-CULTIVATED WITH GLASSWORT (*Salicornia europea*) IN ONE LOOP AQUAPONIC SYSTEM

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## Introduction

Climate change, with its implications for agriculture and aquaculture, is the greatest environmental, economic, and social challenge of our time. In this context, aquaponics is an innovative and environmentally friendly farming system combining aquaculture and hydroponics, following circular economy principles (Goddek et al., 2015). In this study, we used European sea bass (*Dicentrarchus labrax*), a species of high commercial value that occupies an important place in Mediterranean aquaculture. It is resistant to changes in temperature and salinity (Yilmaz et al., 2020). Glassort (*Salicornia europea*) is an indigenous species of the Messolonghi lagoon, a Halophyte with tolerance to salinity changes, commercial value, and economic exploitation is recommended (Castagna et al. 2022). The aim of the present study was to investigate the effect of temperature on the food consumption of European sea bass when co-cultivated in a recirculated aquaponic system in combination with glasswort.

## Materials and Methods

The experiment was carried out in the Aquarium Laboratory of the Department of Fisheries and Aquaculture of the University of Patras in Messolonghi (West Greece). The experimental protocol was approved by the Ethics Committee of the University of Patras. Three autonomous aquaponic systems with a total volume of 432.2 L were used. Each aquaponic system consists of three cylindrical tanks of an 87.8 L/tank volume and two NFT (nutrient lmfilm technique) hydroponic subsystems of an 18.8 L total volume. Each aquaponics system was supported by a 150 L total volume filter, consisting of a mechanical, a biological, and a sump filter. The flow rate of the filter was designed to be upflow and downflow through the filter bed.

Three treatments took place based on temperature, namely at 20$^{o}$C, 25$^{o}$C and 30$^{o}$C, respectively. The temperature of 20$^{o}$C was selected as the optimum temperature for sea bass growth, 25$^{o}$C as the average temperature, and 30$^{o}$C as the maximum temperature for sea bass growth (Yilmaz et al. 2020). In total, 135 European sea bass with an average initial weight of 2.22 ± 0.06 g and an average initial length of 6.21 ± 0.07 cm were used (15 individuals per rearing tank). 24 salicornia plants were distributed in 6 sub-hydroponic subsystems/aquaponic systems. The duration of the experiment was 75 days, with a feeding ratio of 5% of body weight, three times per day. Abiotic parameters were measured every two days using a multimeter (HACK 40QH) to measure pH, nsalinity and temperature and a spectrophotometry analyzer (HACH3800) for TAN, $NO_2^-$, $NO_3^-$ and $PO^{4-3}$). At the end of the experiment, seabass final body weight, food consumption (FC) and specific growth rate (SGR) were calculated., The final height of the plants was also measured.

## Results and Discussion

At the beginning of the experiment, there were no significant differences between the initial boby weight and length of the European seabass and the initial height of glasswort between different temperature treatments (Table 1, ANOVA $p$>0.05). The results showed that an increase in temperature had a positive effect on food consumption and fish growth. Sea bass reared at 25 °C and 30 °C had higher FC and significantly higher SGR compared to those reared at 20 °C (ANOVA, p<0.05, Table 1), confirming that increasing temperature improves fish metabolism (Islam et al., 2020). Glasswort showed the lower growth in the 25°C treatment. In conclusion, a temperature of 25 °C is considered to be optimal for the balanced growth and FC of European sea bass, enhancing this way the performance in aquarium rearing systems under controlled climate change conditions (Goddek et al., 2015).

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**Table 1**. Growth performance and Food consumption of European sea bass (*D. labrax*) and Glasswort (*S. europea*).

| | 20ºC | 25ºC | 30ºC |
|---|---|---|---|
| Initial Body Weight (g) | 2.22±0.06[a] | 2.22±0.05[a] | 2.22±0.06[a] |
| Final Body Weight (g) | 11.15±0.41[a] | 19.97±0.96[c] | 17.18±1.05[b] |
| Specific Growth rate (SGR, %/day) | 2.14±0.06[a] | 2.88±0.06[b] | 2.65±0.10[b] |
| Food Consumption (FC, gr/day) | 2.67 ±0.09[a] | 2.98 ± 0.06[b] | 2.89 ± 0.03[b] |
| Initial Plant Height (cm) | 13.43±1.17[a] | 12.7±1.34[a] | 13.56±0.93[a] |
| Final Plant Height (cm) | 20.15±1.21[b] | 18.95±1.56[a] | 21.4±1.24[b] |

Data were expressed as mean ± S.E.M. Means in a row followed by the same superscript are not statistically significantly different (ANOVA, $p>0.05$).

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# CURRENT BARRIERS, A NOVEL METHOD TO OPTIMIZE CURRENT SPEED IN FISH FARMING CAGES

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## Introduction

Salmon farming is an important industry in the Faroe Islands with a large production in a limited number of sites. The available sheltered farming sites are already in use and production has expanded into highly exposed sites. Further expansion is difficult and will most probably be in highly exposed sites. There has lately been a high focus on open ocean, but there are also possibilities in high current sites if the current speed inside the cages can be reduced to a suitable level for the farmed fish. In the Faroe islands salmon is farmed with high success at sites experiencing tidal currents up to 104 cm/s (measured currents). This is slightly higher than the reported maximum swimming speed of salmon from research (Hvas etal., 2021) even when the current reduction from the cagenets is included. Therefore, it is not expected that sites with much higher currents can be used with conventional equipment.

CFD (Computational Fluid Dynamics) is used to model flow through fish farming nets and the effect the nets have on the flow (Patursson, 2008 and Patursson etal., 2010). The present work presents the first phases of the project "Fish farming in Vestmannasund", where the use of current barriers is investigated in order to enable fish farming in high current sites previously not suitable for farming. The presented phases include 1) finding a suitable site, 2) using CFD to investigate a possible farm and current barrier design, and 3) the test of a small current barrier at the site to test the basic concept.

## Materials and methods

Initial measurements are used for description of the site and for boundary conditions for the CFD model. The measurements are from a bottom mounted ADCP and a boat mounted ADCP. Bottom mounted ADCP measurements provide timeseries of currents at a single location for statistical analysis, while boat mounted currents provide a transect along the boat path. By repeating the same path for just over 12 hours in a semi diurnal tidal site, an overview of the currents at the site through a tidal cycle can be provided.

CFD is used to investigate a full-size farm with a current barrier and a small current barrier. The CFD investigation is performed using steady state RANS, k-ω SST turbulence model and standard wall functions. The boundary conditions are based on the boat mounted current measurement at the time of the highest current speed at the site. The porous media coefficients to describe the nets are based on Patursson (2008). The coefficients for the cage nets are from Patursson etal., (2010), while the coefficients for the current barriers are chosen to provide a suitable current reduction.

The small current barrier was designed to facilitate field measurements of current reduction, mooring force and the vertical movement of the bottom of the current barrier. It was made from two barriers, each barrier was a flat net panel oriented across the flow, one downcurrent from the other.

The current reduction effect of the small current barrier was documented by measuring current upcurrent and downcurrent of the current barrier using two methods 1) One floating ADCP was moored on each side of the current barrier and 2) a boat mounted ADCP was used to measure current transects upcurrent and downcurrent of the barrier. In addition, mooring force in the major current direction was measured and the vertical position of the bottom of the barrier was measured using pressure sensors. The measurements were used to validate the CFD model and to evaluate the effect of the current barrier.

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## Results

The site experiences strong currents. The strongest currents (maximum during the measurement period is 220 cm/s) are aligned along the line from 280 to 100 degrees. Currents are fairly strong in the opposite direction but are low in other directions. Waves are expected to be low in the area, less than 2.5 m Hs.

The CFD results show that a current barrier, designed as a flat net across the flow slightly larger than the cross section of the cages, can shelter a row of at least 5 cages at the present site. The CFD results for the small current barrier show that the reduction is similar to the large barrier, and the wake is clear and long enough to be recognized in boat mounted ADCP measurements. The CFD work also shows that it is difficult to use measured current profiles from a boat mounted ADCP as inlet boundary conditions.

The measurement of current reduction with moored ADCPs gave the possibility to reevaluate the drag coefficient and the porous media coefficients of the net and the drag force gave another independent evaluation. The CFD results of the small barrier were validated against measurements with the boat mounted ADCP and showed good agreement.

The present work shows that it should be possible to have salmon farming in such high current sites as described above using current barriers. The present work also shows that the methods used for modeling and dimensioning were adequate to describe the small barrier and the effect of it. After the presented work was finished, the project has continued, and the farm is now in production. The next step is to improve the design of current barriers and to design current barriers that can withstand the forces from large waves as well. There is also ongoing work to investigate the difference between using steady state RANS and more detailed models like DES.

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# INTEGRATED STABLE ISOTOPE, MULTI-ELEMENTAL, AND FATTY ACID ANALYSIS FOR AUTHENTICATION, TRACEABILITY, AND QUALITY ASSESSMENT OF GILTHEAD SEABREAM (S*parus aurata*)

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**Introduction**

Aquatic products are highly desirable in the human diet, as they provide essential omega-3 fatty acids, high-quality proteins, vitamins, and minerals. However, the growing commercialization of products derived from fisheries and aquaculture creates increasing opportunities for misrepresentation and fraud. Seafood is among the most vulnerable food categories globally, due to complex supply chains, high economic value, and frequent substitution and mislabeling. Aquaculture continues to grow as a sustainable source of animal protein, but robust tools are needed to verify the origin and production method of farmed and wild products. This study addresses these challenges by integrating stable isotope ratio analysis (SIRA), multi-elemental fingerprinting, and fatty acid profiling to differentiate between wild and farmed Gilthead seabream (*Sparus aurata*), determine geographical origin, and assess product quality based on fatty acid composition and the carbon stable isotope ratios of individual fatty acids. Obtained data are added into the IsoFoodTrack reference database which serves as a centralized repository for storing analytical data, supporting traceability and authenticity verification and food fraud prevention. To evaluate sample classification and discrimination by origin and production method, multivariate statistical models such as Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) and Data-Driven Soft Independent Modelling of Class Analogy (DD-SIMCA) were applied, demonstrating the effectiveness of chemometric approaches in seafood authentication.

**Materials and Methods**

A total of 163 authentic and commercial samples of seabream were collected from various marine environments, including the Mediterranean Sea, Adriatic Sea, and the Atlantic Ocean. The stable isotope ratios of light elements ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) were measured in defatted muscle tissues using IsoPrime100 – Vario PYRO Cube Isotope Ratio Mass Spectrometer (IRMS), while the measurements of oxygen ($\delta^{18}O$) isotopic composition in isolated water from the tissue were determined by IsoPrime MultiFlow system coupled to IRMS. Multi-elemental profiling of 30 elements was conducted by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Fatty acid methyl ester composition was determined by Gas Chromatography-Mass Spectrometry Detection (GC-MSD), while carbon isotope ratios of individual fatty acids were analyzed by Gas Chromatography–Combustion–Isotope Ratio Mass Spectrometry (GC-C-IRMS).

**Results and discussion**

Stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ effectively differentiated wild from farmed *Sparus aurata*, with wild samples showing enriched $\delta^{13}C$ and $\delta^{15}N$ values, consistent with a marine-based diet and higher trophic level. Elemental signatures, complemented the isotopic data and further supported the differentiation of geographic origin and production type. The most powerful variables for distinguishing samples from five different geographical locations were $\delta^{18}O$, K, P, Rb, $\delta^{13}C$, $\delta^{34}S$, Mg, Zn, $\delta^{15}N$, S, Co, Hg, and Se. Classification models using OPLS-DA achieved 94% accuracy for geographic origin and 100% accuracy for production method differentiation. The DD-SIMCA model was used for class modeling and verification of Portuguese-origin samples, achieving 96% accuracy, with 93% sensitivity and 98% specificity.

Fatty acid profiling revealed significantly higher concentrations of long-chain ω-3 polyunsaturated fatty acids (EPA and DHA) in wild seabream, as expected based on their natural diet and previous studies. EPA and DHA levels in wild fish reached 7.4% and 17.1%, respectively, compared to 2.1% and 5.3% in farmed seabream. These differences reflect dietary and environmental influences, including feed composition and production method. In addition, preliminary results from carbon isotope ratios of individual fatty acids suggest further potential for differentiating production origin and identifying biogenetic sources, highlighting the value of combining GC-MSD and GC-C-IRMS for assessing nutritional quality and feeding patterns.

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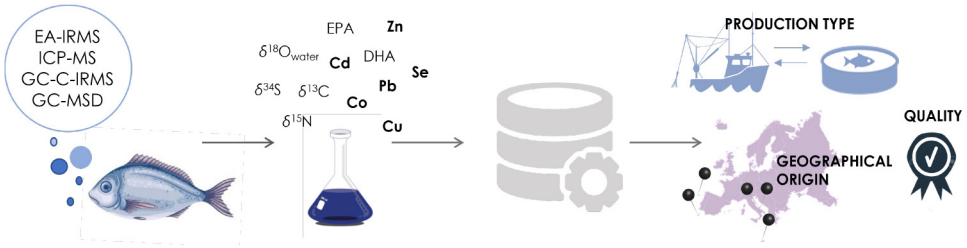


## Conclusion

This study demonstrates the effectiveness of an integrated analytical approach for seafood authentication and quality assessment. The resulting data contribute to the IsoFoodTrack database, supporting the development of reliable traceability systems and reinforcing efforts to prevent seafood fraud across the supply chain.

## Acknowledgements

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# BLOOD BIOCHEMISTRY AND TISSUE HEALTH OF EUROPEAN PERCH (*Perca fluviatilis*) FED DIETS CONTAINING VARYING LEVELS OF *Ganoderma spp.* COMPOSITE MYCELIUM MEAL

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## Introduction

Proper nutrition is essential for maintaining fish health, supporting immune function, and enhancing aquaculture performance. With the increasing need for sustainable alternatives to fishmeal and soybean-based ingredients, novel feed additives are gaining attention. Composite mycelium meals (CMM) derived from Ganoderma spp. through solid-state fermentation (SSF) represent a promising innovation. Ganoderma spp. are rich in functional compounds like polysaccharides and triterpenoids, which may support fish health. While their potential has been demonstrated in species such as tilapia and carp, data on European perch (Perca fluviatilis), a key European aquaculture species, remain lacking. This study investigated the impact of dietary inclusion of Ganoderma-based CMM on blood biochemical parameters and tissue histology in juvenile European perch.

## Materials and methods

A 12-week feeding trial was conducted with juvenile European perch fed one of four diets: a control diet (CMM-0) and three experimental diets containing 5% (CMM-5), 7.5% (CMM-7.5), and 10% (CMM-10) Ganoderma spp. CMM. At the end of the trial, blood samples were collected via caudal venipuncture for biochemical analysis. Serum parameters included cholesterol (CHOL), alkaline phosphatase (ALP), phosphate (PHOS), and total protein (TP), among others. Data were analyzed using linear models in R, with estimated marginal means computed via the emmeans package.

Liver and mid-intestine samples were fixed in 10% buffered formalin, processed using standard paraffin embedding, and stained with hematoxylin and eosin (H&E). Histological sections were assessed using a semi-quantitative scoring system (0–4) to evaluate tissue alterations across five categories.

## Results

All fish fed CMM diets exhibited significantly higher cholesterol and alkaline phosphatase levels compared to controls. Phosphate levels were significantly elevated in the CMM-10 group when compared to the control group. Total protein was lowest in CMM-10 but differed significantly only in comparison to CMM-5. Other biochemical markers, including glucose, creatinine, and liver enzymes, showed no significant differences and remained within physiological ranges.

Histological analysis revealed no consistent or dose-dependent trends in tissue alterations across treatment groups.

## Conclusion

Diets containing Ganoderma spp.-based CMM influenced several blood biochemical markers, suggesting a systemic physiological response, particularly at higher inclusion levels. Elevated cholesterol and visceral fat (data not shown) may point toward shifts in lipid metabolism, what requires further investigation. The absence of major histopathological changes suggests that Ganoderma-based CMM may be safely included in perch diets up to 10%. From a practical perspective, moderate inclusion levels (5–7.5%) may offer a beneficial balance between functional effects and physiological stability. These findings support the potential of mycelium-based ingredients as sustainable feed additives in European aquaculture.

# CHARACTERIZING SKELETAL ONTONGENY AND ANOMALIES IN BALLAN WRASSE (*Labrus bergylta)* LARVAE

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## Introduction

Challenges related to sea lice prevention and treatment remain major cost drivers in salmon farming (Abolofia et al., 2017). The deployment of cleaner fish, such as ballan wrasse (*Labrus bergylta*), has been used as one of several strategies to prevent and control sea lice infestations. Skeletal anomalies are amongst the most common challenges in farmed ballan wrasse (Fjelldal et al., 2021) compromising fish welfare by altering feeding and swimming behavior and increasing stress susceptibility (Boglione et al., 2013). These anomalies have multifactorial causes and are often linked to inadequate early nutrition (Lall and Lewis-McCrea, 2007; Fang et al., 2024). During the weaning phase, it is therefore crucial to use appropriate diets tailored to the species' nutritional needs. This study aimed to better understand the impact of early nutrition on growth performance, osteogenesis, and skeletal anomaly incidence in ballan wrasse larvae, using bone staining and comparative transcriptomic analyses.

## Material and methods

Ballan wrasse larvae (Mowi Stord, Norway) at two days post-hatching (dph) were stocked at a density of 115 larvae.$L^{-1}$ in 200 L tanks at SINTEF/NTNU SeaLab (Trondheim, Norway). Larvae were reared with a 24L:0D photoperiod, oxygen saturation was maintained above 90 %, salinity at 34psu and temperature was gradually increased from 11.8 ± 0.2 to 15.7 ±0.1 °C. The experiment was conducted in triplicates with four experimental feeding regimes differing in the weaning onset. The live feed protocol included *Acartia tonsa* copepodites (NII-III, 160–210 µm) from 4 to 12 dph, rotifers (*Brachionus ibericus*), enriched for 24 hours with Larviva Multigain (Biomar, Denmark), from 10 to 22 dph, and large barnacle nauplii (Cryo-L; *Semibalanus balanoides* NI, 320µm) from 18 to 44 dph. A formulated diet (WINWrasse, SPAROS) was introduced at 5, 17, 24 and 32 dph for Protocols 1, 2, 3 and 4, respectively. From 45 dph onwards, larvae were fed exclusively with the formulated diet. Larvae were sampled from each tank at 4, 16, 23, 31, 45 and 56 dph to determine standard length (SL), dry weight (DW), carbon and nitrogen content (CN), specific growth rate (SGR) and daily weight increase (%DWI). For gene expression analysis, 5-10 larvae per tank were collected at 16, 23, 31, 45, and 56 dph for sequencing. For the analyses the R package glmmSeq (Rivellese et al., 2022) was used to fit negative binomial mixed-effects models at the individual gene level. Additionally, 6–10 larvae per tank were sampled at 31, 45, and 56 dph for assessment of osteogenesis and skeletal anomalies, using a modified double-staining method (Gavaia et al., 2000) and an adapted scoring system (Sæle et al., 2004; Sørøy, 2012; Sæle et al., 2017).

All procedures complied with the Norwegian Animal Welfare Act of 20 December 1974, No. 73 (Sections 20–22, amended 19 June 2009). The experiment was approved by the Norwegian Food Safety Authority (FOTS ID 30573, reference number 23/243983).

## Results and Discussion

At 56 dph, significant differences were observed in DW between Protocols 3 and 4 ($p<0.05$; One-way ANOVA). A notable peak in mortality occurred across all groups between 21–26 dph, with Protocol 1 showing significantly higher cumulative mortality than Protocol 2 at 24 dph ($p<0.05$; One-way ANOVA). Growth patterns up to 30 dph were consistent with previous studies (Sørøy 2012; Høyland 2015) and both growth and mortality followed the tendency described by Malzahn et al. (2022) for a group fed copepod and Cirripedia nauplii. Possible contributing factors to the mortality peak may include appetite fluctuations, environmental conditions, and pathogen presence.

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Urostyle flexion was observed from 6.3 ± 0.10 mm SL and fully ossified vertebrae in larvae measuring 7 mm SL, which was consistent with previous studies in ballan wrasse larvae (Sørøy 2012; Høyland 2015; Malzahn et al., 2022). Protocol 4 had significantly more fully ossified vertebrae ($p < 0.05$; One-way ANOVA) compared to Protocols 1 and 2. At 45 dph, the most common anomaly in Protocols 2 and 3 was abnormal arches and/or spines (A/S) and lordosis was most prevalent in Protocol 1 (41.1%). In Protocol 4, twisted spines was the most frequent anomaly (30.3%) at 45 dph and increased across all Protocols by 56 dph. This increasing trend aligns with previous studies (Sørøy 2012; Høyland 2015), suggesting that twisted spines develop in later stages of ossification. The higher prevalence of this anomaly in Protocol 4 at 45 dph may be linked to its more advanced vertebral ossification at that time point. While anomalies in arches and spines are often not considered severe due to their minimal impact on external morphology, severe cases of spine anomalies can negatively impact fish performance.

Transcriptomic analysis revealed a significant upregulation of genes involved in the oxidative stress response, particularly in Protocol 1. Similar trends were observed for genes involved in the activation and response of the immune system against microbial infections, innate and adaptive immune response and stress resistance. We hypothesize that the introduction of formulated diets altered the bacterial load and community composition, thereby activating antibacterial immune responses.

**Conclusion**

In conclusion, the results of this trial emphasize the potential for refining rearing protocols for ballan wrasse larvae by incorporating advances in nutrition, environmental management, and developmental biology to enhance survival, growth, and skeletal development. Furthermore, we highlight the importance of better understanding ossification types and skeletal anomaly prevalence and integrating this knowledge with improvements in early-life-stage nutrition and longitudinal studies.

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# MODULATORY EFFECTS OF TRYPTOPHAN SUPPLEMENTATION ON NEUROENDOCRINE AND MUCOSAL IMMUNE RESPONSES IN VACCINATED EUROPEAN SEABASS

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**Introduction**
Tryptophan has been shown to play several roles in modulating stress and immune responses in fish, and it is hypothesized that may also serve as a complementary strategy to vaccination in aquaculture. This study evaluates the combined effects of dietary tryptophan supplementation and dip vaccination on skin and intestinal mucosal immunity of European seabass (*Dicentrarchus labrax*), as well as the complex interaction between neuroendocrine-immune systems.

**Materials and Methods**
European seabass juveniles (26.23 ± 7.22 g) were randomly distributed into two different recirculating seawater systems (temperature 20 ± 0.5 °C). In a complete randomized design, fish were fed either a control diet (CTRL) or one of two CTRL-based diets supplemented with tryptophan (TRP1 0.05 % DM and TRP2 0.5 % DM) in triplicate tanks *per* system. After 3 days of feeding, 3 fish *per* treatment from system 1 were euthanized by an overdose of 2-phenoxyethanol and skin mucus and blood (immune status), brain (diced in hypothalamus), pituitary gland, head-kidney (gene expression) and intestine (shotgun proteomics) samples were collected. The remaining fish were dip vaccinated, returning to their original tanks, and sampled after 1 hour, 6 hours, and 21 days post-vaccination. Following vaccination, fish were fed the same diets for 3 more days and after that, all fish were fed the CTRL diet until the end of the trial (21 days). Fish from system 2 were undisturbed following the same dietary regime of system 1.

**Results and Discussion**
Upon vaccination procedures, an expected inflammatory response was observed in fish fed CTRL and TRP2 diets 1 and 6 hours after, as shown by higher plasma cortisol levels, reduced total immunoglobulin M levels, and increased expression of neuroendocrine markers (*htr2a* and *tph1α*). In contrast, this inflammatory response was attenuated in dip-vaccinated fish fed TRP1, as evidenced by low transcription rates of serotonin-related genes, reduced plasma cortisol, and enhanced bactericidal activity in skin mucus - overall displaying a physiological profile similar to undisturbed control fish. Notably, dip-vaccinated fish fed TRP1 and sampled 6 hours post-vaccination exhibited a gut proteomic profile resembling also those of the undisturbed CTRL group. These findings suggest that tryptophan at low supplementation levels may mitigate the stress-related effects of dip vaccination and potentially enhance vaccine efficacy.

Regarding tryptophan supplementation's longstanding effects on fish vaccination efficacy, the lowest supplementation dose counteracted vaccine-mediated reduced gene expression (*c3*, *ido2*, *igm* and *il1β*), again mirroring the gene expression patterns observed in undisturbed fish. This suggests a sustained beneficial effect of tryptophan, even after a short administration period, with effects persisting up to 15 days after the end of the treatment.

**Conclusions**
Overall, the findings highlight a complex interplay between tryptophan supplementation and immune responses, supporting its potential as an effective dietary strategy to enhance vaccination outcomes in aquaculture.

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# DYNAMICS OF *Yersinia ruckeri* INFECTION BY COHABITATION IN JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*): INFLUENCE OF INOCULATION ROUTE AND SHEDDERS-TO-COHABITANT RATIOS

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## Introduction

Infections are common in nature but it is recognised that farming conditions can significantly influence the progression and severity of various diseases in aquatic animals. In this context, the study of host-pathogen interactions is essential to better understand the dynamics of waterborne transmission of pathogens. Enteric redmouth disease (ERM) is a serious septicaemic bacterial infection of salmonid fish species, caused by *Yersinia ruckeri*, a Gram-negative rod-shaped enterobacterium. ERM has a broad host range, widespread geographical distribution, and leads to considerable economic losses in the aquaculture industry. However, few studies focused on the use of a cohabitation mode of ERM exposure, and among them, shedders were intraperitoneally injected (i.p.) fish. In this context, the present study aimed to compare two inoculation routes, a less invasive oral gavage, and the more commonly used i.p. injection, in a ERM cohabitation challenge model using juvenile rainbow trout (*Oncorhynchus mykiss*) as host.

## Materials and Methods

Juvenile rainbow trout were randomly distributed to two separate recirculating seawater systems, each with 8 tanks (50 L water volume), maintained at a constant temperature (20 ± 0.5 °C). Both systems allowed to compare different ratios of shedder (i.e., infected fish) to healthy naïve fish, as well as two different bacterial inoculation routes into shedder fish. The numbers of shedders and healthy naïve fish were adjusted to maintain ratios of 1:3 and 1:5.

Designated shedder fish were anesthetized, marked in the peritoneal cavity using Alcian Blue, and *Y. ruckeri* strain QSP57.1 were i.p. injected or inoculated by oral gavage. In one system, each tank contained 9 or 10 trout (89.5 ± 8.5 g) and among them 3 or 2 fish (1:3 and 1:5, respectively) were i.p. injected with *Y. ruckeri* at a concentration of $7.3 \times 10^8$ CFU *per* fish. In the other system, each tank contained 36 fish (14.6 ± 5 g), with 9 or 6 fish (1:3 and 1:5, respectively) infected through oral gavage with $5.4 \times 10^9$ CFU *per* fish. Dead and moribund naïve fish were sampled for tissue collection (i.e., spleen, anus and gills) in order to quantify bacterial load by molecular methods (i.e., PCR).

## Results and Discussion

Preliminary results showed that different infection routes resulted in diverse onset times for ERM symptomatology. In both bacterial inoculation methods, shedders presented characteristic clinical signs of disease, such as exophthalmia, skin darkening, and subcutaneous haemorrhages around the mouth and throat. Additionally, the higher proportion of shedders-to-cohabitant fish (1:3) led to greater overall mortality compared to the 1:5 ratio in both inoculation routes.

In the oral gavage group, most shedders experienced high mortality within 7–8 days post-bacterial inoculation. Among fish exposed at a 1:3 ratio, 75% of the cohabitant trout survived up to 21 days, corresponding to a 25% mortality rate among naïve fish. In contrast, cohabitants in the 1:5 ratio group exhibited lower mortality, with cumulative losses of only 15% among naïve fish.

In the i.p. group, shedders showed higher and faster cumulative mortality compared to those inoculated by oral gavage with up to 100% mortality within 5 days. In those exposed to the 1:3 ratio, cohabitants showed a 14% cumulative mortality, whereas the their counterparts exposed to the 1:5 ratio, showed a 13% cumulative mortality among cohabitants up to 21 days.

These preliminary findings support the fact that infection pressure and inoculation route play a critical role in pathogen spread and disease dynamics, with increased pathogen load in the system likely facilitating faster and more extensive transmission to naïve individuals. Bacterial quantification in the sampled tissues will bring more insights regarding *Y. ruckeri* propagation during these cohabitation challenge models.

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**Conclusions**

Overall, these findings emphasize the importance of inoculation route and inoculum concentration during *Y. ruckeri* infection by cohabitation. While i.p. injection serves as a robust model for acute disease progression, oral gavage more closely replicates natural transmission dynamics.

Further research is needed to better understand the host immune response during infection by oral gavage inoculation, as this could significantly impact the development of new strategies for disease control and vaccination in aquaculture.

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# POLYCULTURE OF PIKEPERCH (*Sander lucioperca*) AND RUSSIAN STURGEON (*Acipenser gueldenstaedtii*) USING AN ARTIFICIAL COMMON PELLET – IMPLICATIONS ON FEED TO FISH NUTRIENT TRANSFERS IN RECIRCULATING AQUACULTURE SYSTEM (RAS)

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**Introduction**

Aquaculture is crucial for global food and livelihood, with over half of fish for human consumption coming from aquaculture in 2014 (FAO, 2022). By 2030, production is expected to reach 93.6 million tons, a 25% increase. Intensive aquaculture, using Recirculating Aquaculture Systems (RAS), ensures continuous quality fish production. RAS polyculture, cultivating multiple species together, improves resource utilization, waste management, and productivity (Verdegem et al., 2023; Zhang et al., 2011; Zhang et al., 2023). This study examines the impact of using a common pellet in RAS for breeding pikeperch (*Sander lucioperca*) and Russian sturgeon (*Acipenser gueldenstaedtii*), focusing on nutrient transfer efficiency, lipid, fatty acid, and amino acid utilization, and variations in nutrient absorption and flavor profiles.

**Materials and Methods**

All experimental groups were replicated three times. Juvenile pikeperch and Russian sturgeon were reared using pond and intensive aquaculture, respectively. A total of 1224 pikeperch and 576 sturgeons were divided into five groups: monoculture pikeperch (P100), monoculture sturgeon (P0), and three mixed stocks with varying pikeperch-to-sturgeon ratios (P90, P80, P70). Fifteen tanks, each containing 120 fish with an initial biomass of 5.75 kg/m³, were used. Fish were evenly distributed by age, with initial body weights of 18.17±2.65 g for pikeperch and 18.23±3.62 g for sturgeon.

Fish were fed Skretting Europa 15 F floating feed (2 mm pellets), containing 55% crude protein, 16% crude fat, and 4637 kcal/kg digestible energy. Feeding was administered three times daily using automatic feeders. A 12-hour photoperiod was maintained throughout the experiment. The introduction of sturgeon did not significantly affect water quality, demonstrating their compatibility with sustainable aquaculture.

Suspended solids were measured every 28 days, and tanks were cleaned and drained using aquatic nets. Fish were sedated with tricaine mesylate (MS-222) before biometric measurements. Samples, including fish fillets and suspended solids, were collected, filtered, and stored at -18°C for analysis. A total of 45 samples were analyzed using advanced laboratory instruments at the Laboratory of Nutrition.

Amino acid analysis involved protein digestion, derivatization with dansyl chloride, and HPLC-UV detection. Methionine and cysteine were quantified as methionine sulfate and cystic acid, respectively. Lipid extraction was conducted using hexane-isopropanol, and fatty acid composition was determined by gas chromatography. Acid-insoluble ash was used to assess lipid digestibility, providing a reliable alternative to traditional methods.

**Results**

The amino acid profile of pikeperch fillets in monoculture and polyculture with Russian sturgeon showed that lysine reserves were lower in polyculture, while methionine reserves increased. Non-essential amino acids like glycine and proline were highest in the P90 polyculture combination. Fillet lipid content and fatty acid profiles did not show significant changes between monoculture and polyculture. The DHA to EPA ratio almost doubled in flesh compared to food across all groups. Lipid digestibility of the common pellet was excellent, with the highest lipid-ADC in the P70 polyculture tank. EPA and DHA content in pikeperch fillets were lower than in Russian sturgeon, with no significant difference between monoculture and polyculture treatments.

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For Russian sturgeon, no significant changes in lysine or methionine reserves were observed between monoculture and polyculture. The fillet fat content increased significantly in polyculture, with higher MUFA oleic acid content. Lipid digestibility was excellent across all groups, with the highest lipid-ADC in monoculture tanks. EPA and DHA content in Russian sturgeon fillets were higher than in pikeperch, with the highest content in the P70 polyculture tank.

**Discussion**

The study examined the effects of polyculture on the amino acid and lipid profiles of pikeperch and Russian sturgeon in RAS. Key findings highlight the nutritional value, flavor, and texture of the fish. Acid-insoluble ash was found to accurately predict lipid digestibility, offering a reliable alternative to traditional methods. Pikeperch in polyculture showed decreased lysine and increased methionine reserves, while sturgeon amino acid profiles remained stable. Lipid content in pikeperch fillets was unchanged, but sturgeon fillets had higher fat content in polyculture. Lipid digestibility was excellent across all groups, with sturgeon showing robust lipid digestion. DHA and EPA content in pikeperch was similar in both monoculture and polyculture, while sturgeon in polyculture had higher MUFA and DHA content. The study demonstrates the benefits of RAS polyculture in enhancing nutrient utilization and improving fish nutritional quality. Further research is needed to explore the effects of polyculture on fish growth, health, and environmental sustainability in RAS systems.

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# SUSTAINABLE AMMONIA MANAGEMENT IN AQUACULTURE USING CLAY MINERALS

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## Introduction

Attapulgite is a natural clay mineral used in the industry of animal feed (EU 2023/1699) as an anti-caking and binding agent for mycotoxins and other substances. At the same time, it can perform zootechnical functions, affecting the gastrointestinal flora and improving the digestibility of feed in animals. It has been observed that it contributes to the reduction of ammonia during the composting process and effectively removes it from water (Xie et al., 2012, Teng et al., 2024). The purpose of this study was to evaluate the effectiveness of attapulgite in reducing ammonia excretion in the aquatic environment during fish farming.

## Materials and methods

All procedures followed the EU Directive 2010/63/EU for animal welfare and were conducted in licensed facilities (EL-43BIO/exp-01) at the Aquaculture Laboratory, University of Thessaly, Greece, by accredited scientists (FELASA, Functions A–D). 954 gilthead sea breams (*Sparus aurata*, B.W. 2.63 ± 0.46 g) were distributed into 18 aquaria (250 L; 53 fish each) with stable water conditions (salinity 33 ppt, temperature 21 ± 1 °C). Two experimental feeds were used: (1) a control diet and (2) a diet supplemented with attapulgite and 3% activated carbon (3 kg/ton). Six groups (three fed at 4% biomass and three fed *ad libitum*, each in triplicate) were constituted, and the biological filter in the recirculation systems was activated as shown in Table 1.

Fish were fed once daily, six days per week, for two weeks. Ammonia ($NH_3/NH_4^+$) and nitrite ($NO_2^-$) were measured daily at three time points: pre-feeding (PR-F), 2 h post-feeding (2h PST-F), and 9 h post-feeding (9h PST-F), using API commercial assays. No mortalities or injuries occurred. Data were analyzed separately for each feeding method using ANOVA (SPSS 26, $P < 0.05$).

## Results and discussion

In the case of feeding *ad libitum*, under conditions of inactivation of the biological filter, it was observed that the presence of attapulgite contributed to maintaining ammonia levels at significantly lower concentrations compared to the control group that received food without attapulgite (Fig. 1. B). Furthermore, these ammonia concentrations did not show statistically significant differences compared to those of the group in which the biological filter remained fully operational (Fig. 1. B). In contrast, the addition of attapulgite, in the absence of a biological filter, did not affect the levels of nitrite ions, which remained unchanged compared to the control group (Fig. 1. D). Attapulgite is a promising clay mineral with a wide range of applications in aquaculture and the aquatic environment, offering significant prospects in reducing the negative effects of ammonia on fish health. This readily available and economical material has a surface with excellent absorption capacities, acting catalytically in the degradation of ammonia (Xu et al., 2023).

**Table 1. Experimental groups and treatments**

| Experimental groups | Feeding | Biofilter | Experimental diet |
|---|---|---|---|
| CONTROL F-OFF | 4% biomass / *ad libitum* | OFF | CONTROL |
| ATTAPULGITE F-OFF | 4% biomass / *ad libitum* | OFF | ATTAPULGITE |
| ATTAPULGITE F-ON | 4% biomass / *ad libitum* | ON | ATTAPULGITE |

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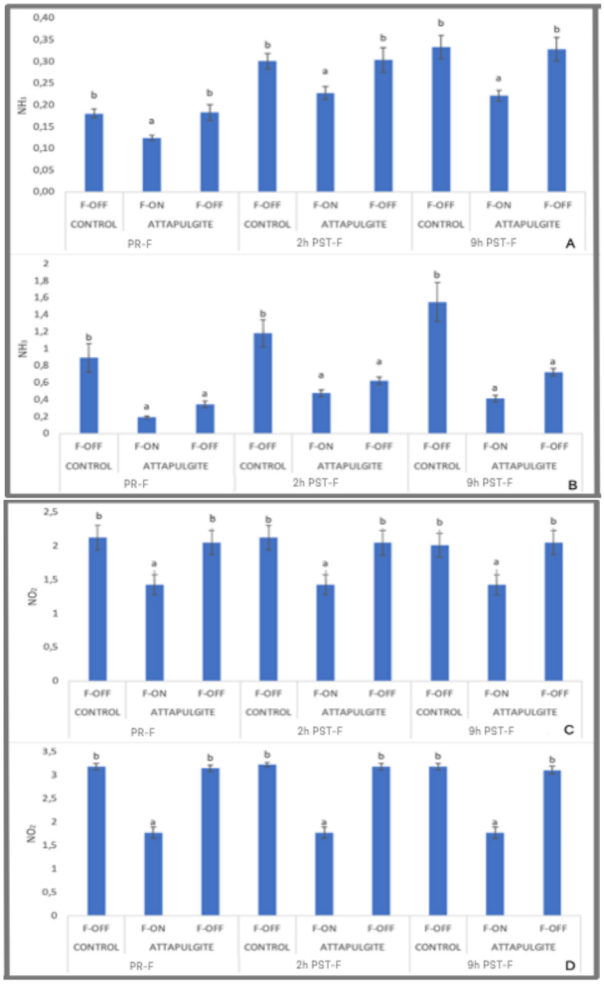


Figure 1. $NH_3$ and $NO_2^-$ values (mean ± standard error, A. C: 4% biomass, B. D: *ad libitum*) in treatment groups, measured before feeding, 2 hours post-feeding, and 9 hours post-feeding. Different letters indicate statistically significant differences among means within the same time point ($P < 0.05$). The absence of letters denotes no statistically significant differences between the tested groups.

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# REDOX-DRIVEN VALORISATION OF MARINE RAS SLUDGE AND EFFLUENTS FOR NUTRIENT RECOVERY AND *ULVA SP.* CULTIVATION

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Recirculating aquaculture systems (RAS) offer a solution to counter eutrophication risk through the treatment or recycling of nutrient-rich effluents. Applying the residual resource concept improves these effluents by adding value through recovery and recirculation of nutrients. The objective of this study was to determine the optimal solubilization oxidation-reduction (redox) conditions for increased solubilization of nutrients produced from marine RAS sludge. Thus, increasing its bioavailability for seaweed development and optimizing the utilization of sludge. The solubilisation of relevant nutrients as nitrogen (N), phosphorus (P), iron (Fe), and manganese (Mn) were examined under three redox potentials: aerobic, intermittent aeration and anaerobic. The results showed that anaerobic conditions led to the highest solubilization degree (0,55 ± 0,06 g sCOD/g TCOD) compared to aerobic (0,45 ± 0,07 g sCOD/g TCOD) and intermittent aeration (0,34 ± 0,09 g sCOD/g TCOD). The optimum fermented sludge supernatant (FSS) obtained was subsequently mixed with RAS overflow water (OW). This mix was evaluated against two conditions: FSS+OW with trace elements (TE) and OW alone, in a 10-day growth trial for assessment of both Ulva sp. specific growth rate and nutrient uptake efficiency. In this test, Ulva cultivated with FSS+OW presented the highest specific growth rates (SGR) (9,07 ± 1,49 %/day) than those treated with FSS+OW+TE (6,71 ± 1,95 %/day) and OW alone (4,95 ± 1,11 %/day). This indicates that the supernatant derived from anaerobically treated marine RAS sludge, when mixed with OW, can stimulate Ulva growth more effectively than the mix supplement with TE and OW alone. While previous research has demonstrated the feasibility of using RAS water for seaweed growth, the current study indicates the role of sludge in supplying nutrients to OW, increasing seaweed growth. The sludge utilisation could reduce the reliance on chemical trace element supplementation in seaweed farming, enhancing sustainability and at the same time reduce the costs of saline sludge treatment. These findings highlight the potential of valorising marine RAS effluents as a sustainable nutrient source in a circular bioeconomy for seaweed production.

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# LONG-TERM COMPARISON OF GREEN AND RED MACROALGAE EXTRACTS AND β-GLUCANS ON THE DISEASE RESISTANCE, GILLS INTEGRITY AND GROWTH PERFORMANCE OF NILE TILAPIA

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This study aimed to evaluate the efficacy of green and red macroalgae extracts and β-glucans on the disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*) in long-term dietary use. An algae-based marine sulphated polysaccharides (MSP) extracts, from Ulva sp. and Solieria chordalis, and 60% purified yeast-based β-glucans available in the market were used in the feeding trial. Fish weighting ~4 g were reared at 26-28 °C, and average dissolved oxygen 6 mg/L. Fish were assigned to 4 different dietary treatments and 6 replicates (tanks of 60 L): Control; Algae MSP 0.3%; β-Glucan A 0.1% and β-Glucan B 0.1%. Fish were stocked at a density of 15 fish/tank (90 fish/treatment) and fed twice a day for 110 days. At the end of the feeding period, all fish were weighed to assess growth, and 8 fish per treatment were sampled for innate immune response analysis. Another 6 fish per treatment were exposed via immersion to a bacterial solution containing *Aeromonas hydrophila* and *Streptococcus agalactiae*. Following 24 hours, gill samples were collected for histopathological evaluation using a diagnostic scoring system performed by a specialized laboratory. Additionally, a third group of 12 fish per treatment was intraperitoneally challenged with the same bacterial solution, and survival was monitored for 7 days. Data were analyzed using one-way ANOVA with means compared by Duncan's test ($p<0.10$) or Kaplan-Meier for survival data (SigmaPlot, Systat Software, San Jose, CA, USA). Tilapia fed Algae MSP showed the most consistent results overall: final weight gain was 3.4% higher than the control ($p = 0.071$); lysozyme activity was 109% higher ($p<0.001$); and superoxide anion production (NBT assay) was significantly higher in all supplemented groups compared to the control ($p<0.001$). Myeloperoxidase activity was highest in the β-Glucan B group, while hemolytic complement activity was greatest in the β-Glucan A group. Algae MSP was the only treatment that showed significantly higher survival after bacterial challenge (50% vs. 16.7%) compared to control. Although survival was numerically higher in fish fed β-Glucan A (33.3%) and B (25%), differences were not statistically significant. Gill histopathological scores were 1.15 (Control), 0.17 (Algae MSP), 0.42 *(β-Glucan A), and 0.*30 *(β-Glucan B), representing* a 85% reduction in tissue damage compared to the challenged control. In conclusion, Algae MSP demonstrated the most consistent overall performance, excelling in growth, innate immunity, gills protection, and survival meanwhile β-Glucans showed intermediate effects across evaluated parameters. The results highlighed the algae-based solution as a tool for strengthening natural defenses in aquaculture due to its broader benefits in long-term use.

# LIGHT, NUTRIENTS, AND GROWTH: MODELING *Ulva ohnoi* FOR SUSTAINABLE AQUACULTURE DESIGN

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## Introduction

Macroalgae (e.g. *Ulva* spp.) provide essential ecosystem services as biofiltration through the absorption of dissolved nutrients, such as nitrates. Among them, *Ulva ohnoi* (Linnaeus) is especially distinguished by its versatility, rapid growth rate, and high nutrient uptake efficiency, making it a worthwhile candidate for use in an Integrated Multi-Trophic Aquaculture with water recirculation system (IMTA-RAS). Designing and scaling-up such systems is usually complex and expensive. However, mathematical modeling is a useful tool to make the process easier, helping in the design and system optimization by enabling virtual simulations to identify optimal cultivation conditions and reduce the need for large-scale experimentation.

Here, we developed a mathematical model for *Ulva* growth and nutrient uptake under different light conditions. We carried out three independent batch-culture experiments in multi-well plates, measuring biomass production (dry weight) and nutrient dynamics (nitrate concentration) over time. A set of candidate models was established and evaluated using a multi-experiment parameter estimation approach, global optimization, and various goodness-of-fit criteria.

The selected model, based on Droop kinetics, incorporates nitrogen quota dynamics and explicitly accounts for light intensity effects. It achieves high predictive accuracy ($R^2 > 90\%$; NRMSE = 22%) and is capable of reproducing the observed growth patterns. Due to its strong precision, the model can now be used for simulation and numerical optimization to support the automated design of IMTA-RAS systems tailored to *Ulva* cultivation.

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# GRINNAQUA INSIGHTS FROM STAKEHOLDER ENGAGEMENT: ADDRESSING CHALLENGES AND OPPORTUNITIES IN AQUACULTURE

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The GRINNAQUA project is dedicated to enhancing sustainable aquaculture practices through research, collaboration, and innovation. As part of this initiative, an event was organized, in collaboration with the MycoFish (*PTDC/CVT-CVT/2660/2021*) and AquaClimAdapt (*PTDC/CTA-AMB/0592/2021*) projects to gather together academia, aquaculture industry stakeholders, and policymakers, in equal representation. This event provided a platform for open discussions on both the broad challenges facing the aquaculture sector and the specific topics that are crucial for its future.

The conversations covered a range of topics, from the use of artificial intelligence in aquaculture and the role of functional feeds in fish health to concerns about food safety and animal welfare. Beyond these focused discussions, participants also tackled larger, industry-wide challenges such as sustainability, regulatory pressures, market access, and the need for innovation. A key point of focus was how different policies—from environmentally protective measures to more liberal market approaches—could influence the sector's growth and sustainability, either presenting opportunities or posing barriers.

The findings from these discussions served as the basis for developing position papers and policy briefs, which aim to guide future research and inform policy decisions. This poster summarizes the key takeaways from these events, focusing on the challenges identified, the policy recommendations proposed, and the strategies suggested to help the aquaculture sector grow in a sustainable and competitive way.

GRINNAQUA is co-funded by the European Union (GA 101079467) and the UK Research and Innovation (UKRI). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union, the Research Executive Agency (REA) or the UKRI. Neither the European Union nor the granting authorities can be held responsible for them.

# GRINNAQUA PROJECT: BUILDING CAPACITY FOR A MORE SUSTAINABLE AQUACULTURE

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The GRINNAQUA project, aligned with the EU's Farm-to-Fork Strategy, aims to strengthen CIIMAR's research capabilities and its leadership role in the aquaculture industry. In partnership with leading European institutions— Consejo Superior de Investigaciones Científicas (Spain), the University of Bergen (Norway), and the Roslin Institute at the University of Edinburgh (UK)—one of the project's focus is on enhancing expertise in fish health, genetics, and immunology.

A key part of GRINNAQUA's approach falls on its training program, designed to help early-career researchers and professionals to build the skills needed to address pressing challenges in aquaculture. Through a series of hands-on workshops and summer schools, participants have gained valuable insights into cutting-edge techniques and tools, particularly useful for the Aquatic Animal Health field. The learning materials developed during these events include:

- **Workshop 1:** *Theoretical-practical workshop about omics tools in aquaculture* – Led by the University of Edinburgh (UEDIN)
- **Workshop 2:** *Theoretical-practical workshop on fish vaccination* – Led by Consejo Superior de Investigaciones Científicas (CSIC)
- **Workshop 3:** *Theoretical-practical workshop on fish health and welfare in industrial aquaculture* – Led by the University of Bergen (UiB)
- **Summer School 1:** *Genomic and genetic tools for the blue revolution* – Led by UEDIN

These materials—such as presentations, practical guides, and interactive exercises—were designed for easy access and long-term use. They provide essential resources for both academic programs and professional development in the aquaculture sector.

This poster will highlight the key learning materials created throughout the project, showcasing how these resources support the ongoing development of aquaculture professionals and how these newfound skills can contribute to building a more sustainable and resilient aquaculture sector in Europe.

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# ELECTROCARDIOGRAPHIC ASSESSMENT OF ANAESTHESIA IN JUVENILE TAMBAQUI *Colossoma macropomum* USING MENTHOL

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**Introduction**
In intensive aquaculture systems, fish are exposed to stressors that compromise welfare, and the use of anaesthetics is an effective strategy to reduce stress and preserve animal integrity (Abreu & Urbinati, 2009; Diniz & Honorato, 2012; Ross & Ross, 2008; De Souza et al., 2019). Synthetic compounds such as MS-222, benzocaine, and 2-phenoxyethanol present restrictions and adverse effects (Inoue et al., 2003; Sladky et al., 2001), encouraging the search for natural alternatives such as menthol ($C_{10}H_{20}O$), which is effective, accessible, and widely available (Façanha & Gomes, 2005). However, there is a lack of physiological data on its use (Cantanhêde et al., 2021; Da Costa et al., 2022), and electrocardiography (ECG) is an essential tool for assessing its safety (Ross & Ross, 2008; Da Fonseca et al., 2024). The tambaqui (*Colossoma macropomum*), the most farmed native species in Brazil (Valenti et al., 2021), requires such evaluations; therefore, this study investigated the effect of menthol (100 mg/L) on electrocardiographic activity to validate its safe use.

**Material and Methods**
Twenty-seven juvenile tambaqui (*Colossoma macropomum*) with a mean weight of 23.9 ± 4.2 g were distributed into three treatments: control, ethanol, and menthol 100 mg/L. Fish, sourced from a commercial farm, were acclimated for 30 days at LAET/IFPA – Campus Castanhal, in recirculating water systems with 250 L tanks, controlled photoperiod (12 h light/12 h dark), commercial feed (34% CP), and water quality maintained within recommended levels for the species. Fish were fasted for 24 h before trials. Menthol crystals (98% purity) were diluted in 70% ethanol at a 1:9 ratio, and the concentration of 100 mg/L was chosen based on previous studies indicating efficacy for the species (Cantanhêde et al., 2021; Da Costa et al., 2022). The experimental protocol was approved by the IFPA Animal Ethics and Welfare Committee (No. 4205150421).

ECG recordings followed methodologies from previous studies (Barbas et al., 2017; De Souza et al., 2019; Cantanhêde et al., 2021), using two stainless-steel electrodes positioned ventrally and below the pectoral fin, recording heart rate, QRS duration, and QT and RR intervals for 300 s during induction and recovery. Nine fish per treatment were analysed, each as an experimental unit. Signals were acquired by a high-impedance amplifier, filtered, amplified, and recorded on an oscilloscope inside a Faraday cage. Data normality and homogeneity were verified by Kolmogorov–Smirnov and Levene's tests and means compared using one-way ANOVA followed by Tukey's test, with significance set at $p < 0.05$.

**Results and Discussion**
During anaesthetic induction with menthol (100 mg/L), ECG recordings (Fig. 1) showed that in the first 120 s, heart rhythm and wave amplitudes remained stable and similar to controls ($P > 0.05$), maintaining a typical sinus rhythm. After this period, there was a progressive reduction in heart rate and signal intensity, although sinus rhythm was preserved.

In recovery (Fig. 2), initially low heart rate and amplitude were observed, with transient sinus arrhythmia, followed by a gradual increase in these parameters. However, the 5 min observation period was insufficient for full restoration of baseline cardiac activity. Quantitative analysis (Fig. 3) confirmed a marked decrease in heart rate and prolonged RR and QT intervals at the end of induction, along with partial reversal during recovery, with QRS returning to baseline and QT showing a reduction trend.

These findings are consistent with the expected depressant effect of anaesthetics on the central nervous system and cardiac conduction, as previously reported for compounds such as MS-222, propofol, thymol, and other essential oils (Barbas et al., 2017a; De Souza et al., 2019; Aydın & Orhan, 2021; Cantanhêde et al., 2021). Incomplete recovery within 5 min aligns with observations by Cantanhêde et al. (2021) for higher menthol concentrations. QT prolongation suggests slower ventricular repolarisation, possibly related to transient blockade of L-type calcium channels (Swandulla et al., 1987; Silva, 2020), a known effect in general anaesthesia (Wisely & Shipton, 2002), but reversible and without observed harmful consequences.

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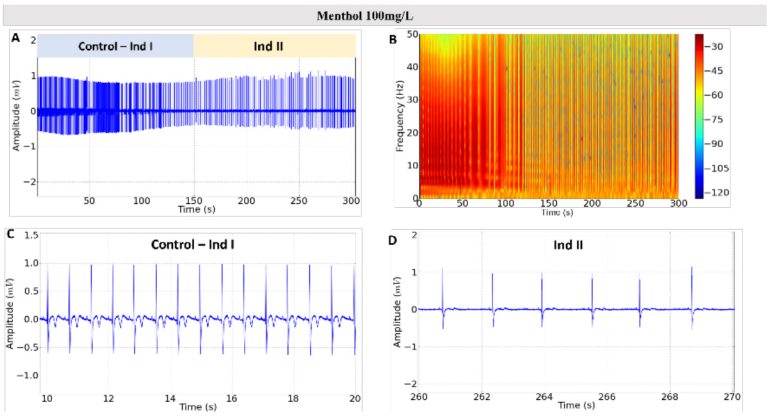


**Figure 1.** ECG of tambaqui (*Colossoma macropomum*) under menthol anaesthesia (100 mg/L): (A) 300 s induction with initial and final stages (Ind I, Ind II), (B) frequency spectrogram, (C) representative trace (Ind I), and (D) representative trace (Ind II).

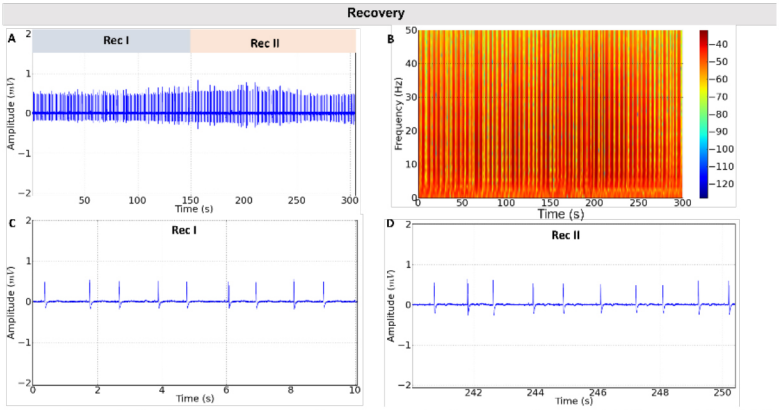


**Figure 2.** ECG of tambaqui during recovery from menthol anaesthesia (100 mg/L): (A) 300 s recording with initial (Rec I) and final (Rec II) stages, (B) frequency spectrogram, (C) 10 s trace at Rec I, and (D) 10 s trace at Rec II.

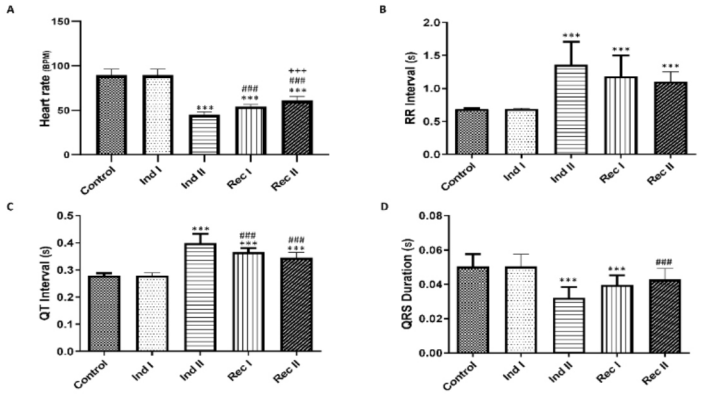


**Figure 3.** ECG parameters during induction and recovery from menthol anaesthesia (100 mg/L): (A) heart rate, (B) RR interval, (C) QT interval, and (D) QRS duration. Symbols indicate significant differences ($P < 0.05$).

## Conclusion

Menthol at 100 mg/L, for up to 5 min, proved effective and safe for juvenile tambaqui, inducing a reversible reduction in heart rate while preserving sinus rhythm and cardiac electrical function. These results indicate its suitability for invasive procedures with minimal risk to homeostasis, reinforcing ECG as a robust tool to validate the safety of anaesthetics in aquaculture.

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# IDENTIFICATION OF TAURINE AS A RESISTANCE-ASSOCIATED METABOLITE AGAINST *Aeromonas salmonicida* AND ITS PROTECTIVE, IMMUNE-REGULATORY, AND MICROBIOTA-SHAPING EFFECTS IN TURBOT (*Scophthalmus maximus*)

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**Introduction**

Infectious diseases represent a major challenge for aquaculture, leading to substantial economic losses and compromising fish welfare. Consequently, identifying viable and environmentally sustainable solutions is a key priority for the sector. Numerous efforts have been made and are continually being undertaken to develop efficient preventive and curative strategies to mitigate the impact of infectious diseases affecting fish while also seeking to avoid, or at least minimize, the use of antibiotics.

An effective approach for the implementation of selective breeding strategies or the development of novel preventive or control measures against infectious diseases involves deciphering the molecular differences between fish full-sibling families that exhibit differential degrees of resistance or susceptibility to a particular pathogen. In this regard, omics technologies, such as genomics, transcriptomics, and proteomics, have proven to be powerful tools to identify a wide range of molecular markers associated with disease resistance. However, the use of metabolomics for this purpose is not so widespread and, to the best of our knowledge, it has not previously been applied to compare the profiles of full-sibling fish families exhibiting different levels of susceptibility to a specific pathogen. This study aimed to identify metabolic markers associated with resistance to the bacterium *Aeromonas salmonicida* subsp. *salmonicida* in turbot (*Scophthalmus maximus*), with the ultimate goal of incorporating resistance-associated metabolites into functional diet formulations.

**Materials and methods**

Untargeted metabolomic analyses of the head kidney and liver from two full-sibling turbot families with differing susceptibility to the pathogen *A. salmonicida* subsp. *salmonicida* were conducted. Metabolites found in higher abundance in the resistant family were preliminarily screened in zebrafish (*Danio rerio*) larvae using an *A. hydrophila* infection model. Based on the promising results, two of these metabolites were incorporated into the diet at a concentration of 1.5% to further evaluate not only their protective effect in turbot against *A. salmonicida*, but also their palatability, impact on growth, and potential histopathological effects. In order to better understand the mechanisms underlying the protection induced by the resistance-associated metabolites, the modulation of the intestinal microbiota (via 16S rRNA amplicon sequencing) and transcriptomic changes (via RNA-Seq analysis) were assessed in response to the functional diet that demonstrated a significant protective effect.

**Results**

A comparative metabolomic analysis was conducted on two full-sibling turbot families with differing susceptibility to the pathogen, revealing a subset of metabolites with differential abundance in the head kidney and liver. Preliminary screening in zebrafish larvae identified 5-L-glutamyl-taurine as a promising protective compound. However, due to the high cost of this metabolite, its precursors—taurine and L-glutamic acid—were tested. Both showed protective effects in zebrafish larvae infected with *A. hydrophila*, leading to their selection for dietary trials in turbot. While dietary supplementation with L-glutamic acid did not significantly affect survival, the taurine-enriched diet significantly improved survival in turbot challenged with *A. salmonicida*, resulting in a 34% higher survival rate compared to controls, despite increased bacterial loads observed at 24 hours post-infection (hpi). Moreover, taurine supplementation had no negative effects on the evaluated parameters.

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Taurine also modulated the intestinal microbiota, reducing alpha diversity through the expansion of *Vibrionaceae* and *Marinobacteraceae*. RNA-Seq analysis of head kidney and intestinal tissues suggested that taurine's protective effect may involve attenuation of the early inflammatory response, potentially minimizing tissue damage. These changes emphasize the complex interplay between diet, immune response, and microbial communities. Overall, this study highlights taurine's potential as a functional dietary additive to enhance disease resistance in aquaculture, while underscoring the value of immunometabolism research in developing strategies to combat infectious diseases.

# THE REGULATION OF REDOX BALANCE UNDERLIES A CONSERVED ANTIVIRAL RESPONSE TO RNA VIRUSES IN FISH

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**Introduction**
Reactive oxygen species (ROS) are byproducts of normal metabolism and play dual roles as both signaling molecules and mediators of oxidative stress. In fish, ROS are generated endogenously, mainly by mitochondria and NADPH oxidases, and influenced by environmental stressors such as pollutants, temperature shifts, and hypoxia. A sophisticated antioxidant system maintains redox homeostasis in fish. Viral infections can disrupt this balance, enhancing ROS production or impairing antioxidant defenses, with consequences ranging from antiviral signaling activation to facilitation of viral replication. The outcome depends on the virus, host, and infection stage. In turbot aquaculture, three major RNA viruses, viral hemorrhagic septicemia virus (VHSV), infectious pancreatic necrosis virus (IPNV), and red-spotted grouper nervous necrosis virus (RGNNV), cause high mortality, economic losses, and lack broadly effective treatments.

**Objective**
Identify conserved host responses in turbot to these three RNA viruses.

**Materials and Methods**
Turbot (*Scophthalmus maximus*) were experimentally infected with VHSV, IPNV, or RGNNV under standardized conditions. At 3 days post-infection, tissues were collected for label-free quantitative proteomics to identify differentially abundant proteins and define common antiviral response against all viruses. Functional assays using head kidney–derived leukocytes were conducted to assess ROS production by macrophages following viral challenge. Pharmacological inhibitors of NADPH oxidase and mitochondrial ROS were applied to evaluate their impact on viral replication in vitro.

**Results**
Mass spectrometry identified 4,499 proteins, with virus-specific sets of differentially expressed proteins (DEPs) in kidney and brain. Only neutrophil cytosol factor 4 (Ncf4) was common to all kidney infections. VHSV predominantly downregulated mitochondrial electron transport chain (ETC) components and the antioxidant superoxide dismutase 2 (Sod2); IPNV caused fewer changes, with modest immune protein modulation; RGNNV triggered the most extensive proteomic shifts, notably upregulating proteins linked to cytoskeleton, translation, metabolism, and ROS detoxification.

Across viruses, mitochondrial and cytoplasmic ROS-related proteins were altered, with VHSV generally reducing and RGNNV increasing their abundance; IPNV changes were milder. Gene expression analyses confirmed virus-specific temporal patterns in ROS-related genes. In vitro, all viruses tended to increase basal ROS production, with varying effects on PMA-induced ROS; in vivo, only VHSV significantly increased ROS in head kidney leukocytes.

Flow cytometry showed virus-dependent modulation of mitochondrial ROS (mitROS), particularly by RGNNV and IPNV. The release of neutrophil extracellular traps (NETs) was enhanced by some infections and interferon (IFN) stimulation, but reduced by IPNV in vivo and RGNNV in vitro. Pharmacological inhibition of ROS affected viral replication in a virus- and inhibitor-dependent manner, confirming ROS as a functional antiviral component.

**Conclusions**
Comparative proteomics of infected turbot revealed a conserved antiviral mechanism centered on ROS production and redox homeostasis.

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Mitochondrial and cytoplasmic ROS pathways are differentially modulated by each virus, with VHSV generally suppressing and RGNNV enhancing ROS-related proteins.

Pharmacological inhibition of NADPH oxidase– or mitochondria-derived ROS alters viral replication, confirming a direct antiviral role for ROS.

Findings provide a molecular basis for designing broad-spectrum antiviral strategies in aquaculture, including antioxidant-based adjuvants or immune modulators.

# EFFECTS OF FISH PROTEIN HYDROLYSATE FROM DISCARDED BLUE WHITING (*Micromesistius poutassou*) ON TURBOT (*Scophthalmus maximus*) GROWTH AND FEED INTAKE

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## Introduction

One of the key challenges in making fish farming more sustainable is replacing fishmeal with alternative ingredients. While plant-based substitutes have been explored, they often fall short due to poor digestibility and amino acid imbalances that hinder fish growth (Hussain et al., 2024). Fish protein hydrolysates (FPH) offer a promising alternative, as their rich amino acid and bioactive peptide content improves growth and feed efficiency (Siddik et al., 2021). In Galicia, Northwest Spain, the fishing industry generates large volumes of discards that can be repurposed into FPH: a cleaner, more sustainable option than traditional fishmeal. This process not only reduces environmental impact but also yields valuable compounds such as proteins, oils, and minerals.

Blue whiting (*Micromesistius poutassou*), a commonly discarded species, has shown potential as raw material for FPH (Vázquez et al., 2024). Based on a previous work (Comesaña et al., unpublished), low molecular weight FPH from blue whiting (LBW FPH) produced comparable results to a commercial diet in turbot (*Scophthalmus maximus*)—a flatfish predominantly farmed in Galicia, Northwest Spain—regardless of the inclusion level (5, 10 and 15%), making it a viable alternative for replacing traditional fishmeal. Given the absence of adverse effects, the present research explores higher inclusion levels, completely replacing fishmeal. Over a three-month period, the effects of replacing fishmeal with 15 and 30% LBW FPH will be evaluated, focusing on growth performance. This approach supports circular and local economy principles and aims to enhance the sustainability and efficiency of turbot aquaculture.

## Materials and methods

Juvenile turbots (*Scophthalmus maximus*) were obtained from a local hatchery (Insuíña S.L., Oia, Pontevedra, Galicia, Spain) and transported to the facilities of the Oceanographic Center of Vigo. Experimental turbots (18.4 ± 0.18 g) were distributed into 9 tanks of 100 L each (n = 60 fish/tank). They were kept under natural photoperiod and a constant temperature of 16ºC. After an acclimation period, the feeding trial began and lasted for 3 months. Fish were fed ad libitum twice a day (9 am and 4 pm) up to a maximum of 3% of their body weight daily. Three different diets were used and tested in triplicate: i) Control with 30% fishmeal + 0% LBW FPH (LBW0), ii) 15% fishmeal + 15% LBW FPH (LBW15), and iii) 0% fishmeal + 30% LBW FPH (LBW30). The following zootechnical parameters were evaluated: daily feed intake, initial and final weight and total length, and liver weight. From these data, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), condition factor, and hepatosomatic index (HSI) were calculated.

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**Table 1.** Growth performance, feed utilization, and biometric indices of juvenile turbot fed with control diet (LBW0) and LBW15 and LBW30 diets containing 15% and 30% respectively, of low molecular weight FPH of blue whiting.

| | LBW0 | LBW15 | LBW30 |
|---|---|---|---|
| **Growth performance** | | | |
| Inicial weight (g) | 18.5 ± 0.4 | 18.4 ± 0.1 | 18.5 ± 0.3 |
| Final weight (g) | 68.6 ± 2.0 a | 63.9 ± 0.7 ab | 57.4 ± 3.2 b |
| Weight gain (g) | 50.1 ± 1.7 a | 45.5 ± 0.5 ab | 38.9 ± 3.1 b |
| SGR, specific growth rate (%/day) | 1.45 ± 0.02 a | 1.38 ± 0.01 ab | 1.26 ± 0.06 b |
| **Feed utilization** | | | |
| Feed intake (% body weight/day) | 2.13 ± 0.12 | 2.45 ± 0.08 | 2.42 ± 0.04 |
| FCR, feed conversion ratio | 1.24 ± 0.11 | 1.78 ± 0.11 | 1.93 ± 0.13 |
| **Biometric indices** | | | |
| Condition factor, K | 2.02 ± 0.04 | 2.09 ± 0.02 | 2.18 ± 0.06 |
| HSI, hepatosomatic index (%) | 1.19 ± 0.04 a | 1.33 ± 0.01 ab | 1.52 ± 0.10 b |

The values are expressed as mean ± SEM of triplicate groups (n = 3). Data were analysed using one-way ANOVA and different letter indicates significant differences between diets ($p<0.05$).

## Results and discussion

Juvenile turbot fed with a diet in which fishmeal was completely replaced by LBW FPH (LBW30) exhibited reduced growth performance compared to control (LBW0), as evidenced by lower final weight, weight gain and SGR. No significant differences were observed in feed intake, FCR or condition factor. Moreover, HSI was significantly higher in fish fed the LBW30 diet compared to LBW0.

These findings suggest that full substitution of fishmeal with the LBW FPH may negatively affect growth in juvenile turbot. Further studies would be required to understand the physiological mechanisms behind the increased HSI and reduced growth, and to assess the broader implications of using this diet. In contrast, partial replacement (LBW15) showed comparable results to the control, indicating its potential as a sustainable alternative that could valorize previously discarded products and contribute to more environmentally friendly aquaculture practices.

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# ANTIMICROBIAL STAINLESS-STEEL SURFACES FOR CONTROLLING *Pseudomonas* spp GROWTH IN FISH-PROCESSING FACILITIES

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## Introduction

The proliferation of *Pseudomonas* species presents a significant challenge in the fish industry, as these bacteria are among the most common spoilage organisms in refrigerated fish products. Capable of thriving at low temperatures, *Pseudomonas* produce enzymes that break down proteins and lipids, resulting in unpleasant odors, texture deterioration, and a markedly reduced shelf life (Ben Mhenni et al., 2023). Their presence not only compromises the sensory quality of fish but also raises serious concerns regarding food safety and economic losses. Therefore, in addition to conventional measures such as strict hygiene protocols, temperature control, and appropriate packaging, innovative strategies are needed to curb *Pseudomonas* growth. One promising approach is the functionalisation of stainless-steel surfaces with compounds that exhibit antimicrobial properties, which could serve as an effective barrier to bacterial contamination along the processing chain. The aim of this work was to study the antimicrobial effect of thymol and eugenol functionalised on stainless-steel plates.

## Materials and methods

In this study, 316L stainless-steel plates treated and functionalised with thymol and eugenol were prepared. These surfaces were evaluated against *E. coli* K12 CECT 433, a reference bacterium, as well as *Pseudomonas aeruginosa* CECT 108, a key bacteria in the seafood industry. A susceptibility test, using the microdilution method, of the studied strains against two essential oil compounds (EOCs), eugenol and thymol, in their free form was first performed to obtain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both EOCs against both strains.

Functionalised plates were qualitatively characterised with Orange II and quantitatively using energy-dispersive X-ray spectroscopy (EDS) coupled to Field Emission Scanning Electron Microscope (FESEM) to confirm the proper anchoring of the compounds.

The antimicrobial activity of functionalised stainless-steel surfaces was evaluated by applying a bacterial inoculum ($10^5$ CFU/mL) on the surfaces. After 5 h of incubation at 30 °C, the microorganisms were recovered and quantified on TSA Petri dishes to determine microbial reduction.

## Results

*P. aeruginosa* was shown to be more resistant than *E. coli* K12 for both compounds, with a MIC of 2 mg/mL for eugenol and 0.5 mg/mL for thymol, while for *E. coli* K12 these values were 0.5 mg/mL and 0.25 mg/mL, respectively.

Orange II and FESEM results confirmed the functionalisation of the steel surfaces with the EOCs.

Furthermore, results showed that functionalised plates were able to reduce the microbial load by up to 4.5 log for *E. coli* K12 for both, thymol and eugenol. *P. aeruginosa* microbial load was reduced by 2.3 log on plates coated with thymol, but no significant reduction was observed with eugenol at our working concentration, due to the high MIC.

## Conclusion

The functionalisation of stainless-steel surfaces with thymol or eugenol has demonstrated high antimicrobial potential, which could contribute to improving food safety in fish processing plants.

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**Acknowledgements**

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# TOWARDS FULL GENOMIC REPRESENTATION OF THE BONY FISH PHYLOGENY

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## Introduction

Bony fish are an extremely diverse group in terms of morphology, evolutionary history and taxonomy, comprising more than 95% of all living fish and half of all known vertebrates. Additionally, this group comprises an important number of highly valuable species for global aquaculture and fisheries. Consequently, bony fish have been the subject of intensive research in multiple fields, including ecology, evolutionary biology, and fish production. The development of genomic sequencing technologies has enabled comparative studies within the group, providing powerful insights into the genetic basis of local adaptation, genome evolution, and the genetic architecture of production traits, knowledge needed for the sustainable management of farmed and wild genetic resources. However, in-depth biological and evolutionary studies require suitable genome assemblies to be available across multiple species and taxonomic groups. Here we provide a snapshot of the genomic resources currently available in the public databases, with a special focus on existing gaps throughout the fish phylogeny.

## Material and methods

The NCBI and Ensembl databases were scanned to record all bony fish species with available genomic data. Information on all available raw sequencing data was also retrieved from the Sequence Read Archive (SRA). Both the nomenclature and the taxonomic classification were taken from the NCBI, and the fish phylogeny by Betancur-R et al. (2017) was taken as reference of the phylogenetic relationships among taxa. The total number of recognized bony fish species was obtained from the Catalogue of Life.

## Results

Genome assemblies were found for a total of 1,744 bony fish species, mostly released in the last five years. Gene annotation has also experienced a boost, although only 16.49% of the current genomes are annotated. At the organism level, species of high commercial value in aquaculture, fisheries and sport fishing, were found to be particularly enriched with genomic data. Despite the accelerated rate of genome sequencing, the number of available genomes still represents only 5.36% of all recognized bony fish species. Nevertheless, they are well distributed throughout most of the bony fish phylogeny, with 93.05% of orders and 52.14% of families being represented. The five orders containing the largest number of assembled genomes are Cichliformes, Perciformes, Siluriformes, Cypriniformes and Cyprinodontiformes, where some of the species with the highest aquaculture production can be found, such as carp, catfish, perch and grouper. However, a fraction of the phylogenetic tree remains unrepresented, particularly within the Percomorpha clade. Despite the lack of fully assembled genomes, 19 out of the 20 gaps have associated high-throughput sequencing data in the SRA repository, so these gaps should not remain for too long.

## Conclusion

We are witnessing a genomic revolution, with exceptional efforts being dedicated to fish genomic resources in both commercial and non-commercial sectors. New sequencing efforts encompassing the full fish phylogeny, fuelled by the constant development of sequencing and assembly technologies, will provide an unprecedented array of genomic resources that can be used to address outstanding biological and evolutionary questions in bony fish.

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# REDUCED HAEMOCYTE VIABILITY AND INCREASED ROS PRODUCTION LEAD TO CELLULAR DAMAGE AND LOWER METABOLIC EFFICIENCY IN SLOW-GROWING PACIFIC OYSTERS

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## Introduction

The Pacific oyster *(Magallana gigas)*, introduced to New Zealand in the 1950s, is central to a major aquaculture industry producing nearly 2,000 tonnes annually. After a decline due to the OsHV-1 µvar virus outbreak in 2010, the industry has been recovering, aided by selective breeding. Growth rate variability among individuals offers both challenges and opportunities for optimization, driven by physiological and genetic factors. Studies have linked growth differences to food acquisition, metabolic costs, and gill morphology—especially surface area and ciliary structure.

In mussels, fast growers have denser ciliary networks and larger gills, while slow growers exhibit elevated antioxidant activity, potentially signalling higher oxidative stress (Pérez-Cebrecos et al., 2022). Immune function is influenced by haemocytes, which are involved in immune defence and other functions like shell formation. Gene expression studies suggest that slow growers may have immune inefficiencies, affecting overall fitness (Prieto et al., 2019). Reproductive energy demands can also influence growth. Triploid oysters, which allocate more energy to somatic growth, provide a model for investigating these trade-offs. Previous field studies suggest that growth-rate phenotypes in triploids correlate with reduced reproductive investment, leading to more efficient somatic growth.

This study investigates the physiological, histological, and immunological aspects of growth variation in M. gigas, exploring how triploidy influences growth, immune function, and oxidative stress. By examining these factors, the study aims to inform selective breeding strategies and improve oyster aquaculture sustainability.

## Materials and Methods

Triploid and diploid *M. gigas* oysters were produced from a biparental cross and reared under identical hatchery conditions. Growth phenotypes were classified into fast and slow growers within each ploidy group. Standard physiological measurements (clearance, ingestion, absorption, and respiration rates) were used to calculate scope for growth. Flow cytometry was applied to assess haemocyte activity, ROS production, and viability. Gill and digestive gland tissues were analysed for antioxidant enzyme activity (SOD, CAT), histopathology, and structural indices. Gametogenic stage was also evaluated histologically. All measurements were size-standardized and statistically analysed to test for effects of ploidy and growth phenotype.

## Results

Fast-growing oysters were 51% heavier and 87% larger than slow growers in both diploid and triploid groups. Clearance rates (CR) were 23% higher in fast growers ($3.60 \pm 0.86$ $L \cdot h^{-1} \cdot g^{-1}$) compared to slow growers ($2.76 \pm 0.48$ $L \cdot h^{-1} \cdot g^{-1}$), while absorption efficiency (AE) was 11% higher in slow-growing oysters ($0.74 \pm 0.06$) compared to fast-growing oysters ($0.66 \pm 0.15$). Scope for growth (SFG) was 37% higher in triploid oysters ($153.06 \pm 47.33$ $J \cdot h^{-1}$) compared to diploids ($96.05 \pm 48.56$ $J \cdot h^{-1}$), with fast growers showing a 51% higher SFG ($166.73 \pm 73.58$ $J \cdot h^{-1}$) than slow growers ($82.38 \pm 22.31$ $J \cdot h^{-1}$). In immune responses, fast-growing diploid oysters exhibited 62% higher reactive oxygen species (ROS) production compared to triploid fast growers, with significant reductions in immune cell viability, especially in slow-growing triploids.

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Catalase activity in the gills of triploid oysters (127.78 ± 9.88 μmol·min$^{-1}$·mg protein$^{-1}$) was 30% higher than in diploid oysters (89.80 ± 6.05 μmol·min$^{-1}$·mg protein$^{-1}$), while superoxide dismutase activity was 15% higher in fast-growing oysters (14.87 ± 1.75 U·min$^{-1}$·mg protein$^{-1}$) compared to slow-growing oysters (12.67 ± 2.03 U·min$^{-1}$·mg protein$^{-1}$).

Histopathological analysis revealed a higher prevalence of rickettsia-like organisms in triploid fast growers (0.88) compared to diploid fast growers (0.10). Slow growers exhibited a significantly lower gill structure index (0.66 ± 0.24) compared to fast growers (1.25 ± 0.23), and triploid oysters were more advanced in gamete development stages. Shell organic content was 27% higher in fast growers (15.31 ± 3.83) compared to slow growers (11.25 ± 3.05), while the shell-to-live weight ratio was 10% heavier in fast growers (0.48 ± 0.02) than slow growers (0.44 ± 0.05).

Discussion

Despite being from the same family and raised under identical conditions, fast-growing oysters were 52% heavier than slow growers, underscoring the importance of physiological traits in growth performance and aquaculture optimization.

Fast growers exhibited higher feeding rates without increased metabolic costs, suggesting enhanced metabolic efficiency, which supports their superior growth. This finding aligns with previous research showing that increased ingestion rates do not always correlate with higher metabolic costs (Babarro et al., 2000). Fast-growing oysters also had superior gill structure, a crucial factor in filtration efficiency. Their enhanced gill morphology likely supports their higher clearance rates and growth potential. In contrast, slow growers showed smaller, less viable haemocytes, and elevated ROS production, indicating compromised immune function and higher oxidative stress. This likely contributes to their lower SFG and reduced metabolic efficiency. The presence of basal cells in slow growers may reflect an increased immune turnover to compensate for the reduced viability of haemocytes.

Interestingly, triploid slow growers showed some compensatory advantages, with better gill structure and higher catalase activity than diploid slow growers. This suggests that triploids may invest more in antioxidant defences and somatic growth rather than reproduction. The higher organic shell content in slow-growing triploids, compared to diploid slow growers, further indicates a trade-off between growth and biomechanical protection. These oysters may allocate more energy to shell strengthening, enhancing resilience at the cost of slower growth.

Overall, the study demonstrates the complex trade-offs between growth, immune function, and oxidative stress. Fast-growing oysters exhibit superior growth, metabolic efficiency, and immune function, while slow growers are hampered by immune dysfunction and oxidative stress. Triploid oysters offer some advantages in immune defence. These findings emphasize the need to consider both physiological and immunological factors in aquaculture management and breeding programs aimed at optimizing oyster performance.

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# IN VITRO DIGESTIBILITY OF AQUAFEEDS SUPPLEMENTED WITH INSECT PROTEIN HYDROLYSATES FOR EUROPEAN SEABASS (*Dicentrarchus Labrax*) REARING

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## Introduction

Fish farming requires a high supply of protein, ranging from 50 – 65% in weight of the diet for larval rearing, decreasing down to 45% for juvenile and adult individuals. The main source is fish meal, mainly obtained from herring, anchovy, and other lean fish species. The increasing demand of fish meal for aquaculture and pork farming has increased the prices of fish meal and oil. In this context, the formulation of novel aquaculture ingredients based on alternative proteins (e.g. insect, plant, biomass) has attached increasing interest over the past decade. Edible insects present variable protein content ranging from 40 – 65% of their dry weight and an amino acid composition equivalent to fish meal. Insect rearing is sustainable and economically feasible, due to their high feed-to-protein conversion rates. Zootechnical feeding trials are required to evaluate the effect of new dietary proteins on fish growth and survival, but they are expensive, time consuming and require high amounts of the dietary components. As an alternative, *in vitro* methods simulate the digestion of aquafeeds at lab scale, employing natural enzymes directly extracted from the aquatic species. Although they cannot fully reproduce the physiology of the fish digestive system, they can be easily implemented and are useful to evaluate the bioaccessibility of aquafeed ingredients.

The aim of this work is to evaluate the in vitro digestibility of different protein sources from insect (*Tenebrio molitor*) defatted meal, sunflower (*Helianthus annuus*) seed meal and microalgae (*Nannochlorosis gaditana*) powdered meals employing an INFOGEST protocol adapted for European seabass (*Dicentrarchus labrax*) as reference aquaculture species. The protein source showing the highest digestibility was then tested as protein ingredient in a commercial fish meal-based diet.

## Materials and Methods

Enzyme extracts were recovered from seabass stomach, intestin and caeca pylori after grinding and homogenization at 4ºC with 1:5 water for 2 h. The aqueous extracts were centrifuged and vacuum filtered, recovering the supernatants for determination of pepsin and trypsin activity. Firstly, protein suspensions from insect, sunflower and microalgae were prepared at 1.8 g of protein·100 mL-1 and heated at 85ºC for 15 min to inactivate endogenous enzymes. These suspensions were subjected to simulated digestion in a 200-mL jacketed rector. The digestion comprised two sequential stages—stomach and intestinal phase— of 2 h, both conducted at 25ºC and pH 2 and 8, respectively. The dried stomach and intestine extracts were added at 20 U of pepsin activity·mg-1 protein and 2 U of trypsin activity·mg-1. The extent of the digestion was evaluated by the pH stat method, using an automatic titration system (Metrohm, Switzerland). The protein source showing the highest digestibility (i.e. attaining the highest degree of hydrolysis after completing 4 h of digestion) was then tested as protein replacement in a commercial fish meal-based diet for European seabass (*Dicentrarchus labrax*). To this end, different levels of fish meal protein were substituted by the alternative protein partially hydrolysed (15% w/w of hydrolysate). Enzymatic hydrolysates were prepared from sustainable protein materials employing a mixture 1:1 of Alcalase and PTN at pH 8, 50ºC and enzyme-to-substrate ratio 3%. Response Surface Methodology was employed to optimize the formulation of the experimental meals, varying protein substitution (25, 50 and 75% w/w), and degree of hydrolysis (DH 10, 15 and 20%) through a factorial 3 x 3 design. The protein digestibility of the formulated diets was evaluated by the final degree of proteolysis attained after simulated digestion, which was estimated by the o-phthalaldehyde (OPA) spectrophotometric assay.

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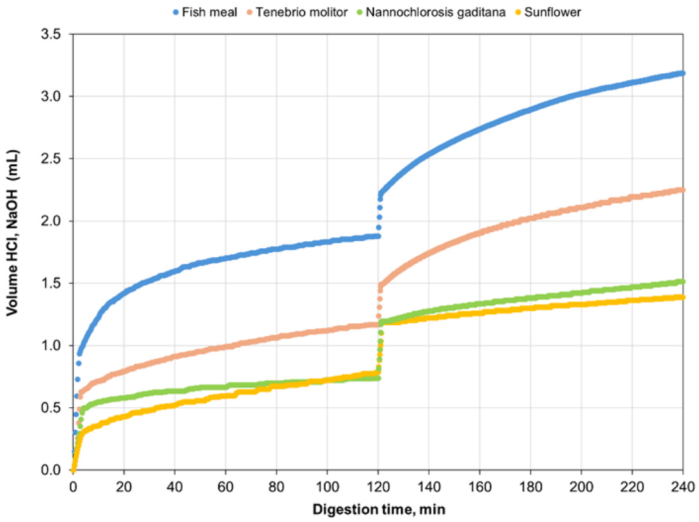


**Figure 1.** Digestion curves of the protein substrates with stomach extract (pH 2, 2 h) and intestine extract (pH 8, 2 h) from European seabass (*Dicentrarchus labrax*).

## Results

Fish meal presents the highest digestibility among the proteins assayed, followed by the insect protein from *Tenebrio molitor*, while sunflower seeds and microalgae were poorly digested. This was attributed to the presence of fibre and other insoluble impurities in the sunflower meal, or the resistance of microalgae membranes against enzyme attack. The digestibility of insect protein was then improved by the inclusion at 15% w/w of its protein hydrolysates. Supplementation of aquafeeds with enzyme hydrolysates improves their digestibility, as well as providing peptides and free amino acids with potential biological activities. A set of experimental diets containing *T. molitor* protein as replacement source were then formulated and subjected to in vitro digestion with European seabass enzymatic extracts. The maximal *in vitro* digestibility was observed for the experimental diet with 25% w/w of insect protein containing 15% w/w of enzymatic hydrolysate at DH 10%. After in vitro digestion, this formulation presented a final degree of hydrolysis of 13.4% (OPA), very similar to that of fish meal (14.2%).

## Conclusion

Insect protein can be used as protein ingredient to replace fish meal in aquaculture diets. Its digestibility can be further improved by partial hydrolysis of the insect meal, as confirmed by the INFOGEST assay adapted for European seabass. The degree of hydrolysis achieved after simulated digestion, which can be determined by the pH stat method or spectrophotometrically, can be used as index to evaluate protein digestibility.

## Acknowledgement

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# ENZYMATIC HYDROLYSIS AND MEMBRANE FRACTIONATION OF SKIPJACK TUNA BY-PRODUCTS TO OBTAIN FUNCTIONAL INGREDIENTS FOR AQUAFEEDS

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## Introduction

Aquaculture industry faces increasing pressure to develop sustainable alternatives to fish meal, driven by the sharp increase in costs and decline in captures of some species intended for fish meal production (i.e. herring, anchovy). In Spain alone, aquafeed consumption reached 181,000 tonnes in 2023, with fish meal as main ingredient (86% w/w of fish meal production is intended for aquafeed).

In this context, by-products from fish industry represent a cost-effective and sustainable feed source. In the case of tuna canning industry, by-products can account for 60-70% of the weight of the processed fish. Heads are the main by-products resulting from tuna canning industry. They present on average 15% w/w proteins, 13.5% w/w lipids, and significant amounts of minerals which makes them suitable for their conversion into aquafeed ingredients. However, their inclusion in aquafeeds is restricted by their poor solubility and digestibility in larvae and juveniles. Digestibility can be improved by the inclusion of variable levels of fish protein hydrolysates. They not only improve the solubility, digestibility and palatability of the aquafeed, but also can display biological activities such as antimicrobial, anti-inflammatory or antioxidant, among others. The supplementation of aquafeeds with antioxidant peptides has a double purpose: (i) extend the self-life of aquafeeds, restraining oxidation of lipid ingredients (ii) help mitigate the oxidative stress generated by the environmental conditions of aquaculture systems.

This study aims to obtain antioxidant peptide fractions from Skipjack tuna (*Katsuwonus pelamis*) heads with application as functional ingredients in aquafeeds. Tuna heads were hydrolysed in an enzymatic reactor and fractionated by sequential ultrafiltration with ceramic membranes of 1, 3, and 8 kDa molecular weight cut-offs. The resulting fractions were tested for their *in vitro* antioxidant activity (i.e. DPPH radical scavenging and ferrous ion chelation). This procedure allows producing peptide fractions with antioxidant activity, which can be incorporated as natural antioxidants into aquafeed diets.

## Materials and Methods

Skipjack tuna (*Katsuwonus pelamis*) heads were obtained from a local canning factory, presenting 38.4% w/w of protein, 9.6% w/w of lipid and 11.5% w/w of mineral matter. The homogenized suspension was fed into an enzymatic reactor, employing two commercial proteases: Alcalase® and Flavourzyme® (Novozymes, Denmark) at pH 7.5, 55ºC and enzyme-to-substrate ratio of 3% w/w.

Tuna head hydrolysates were fractionated employing 3 ceramic ultrafiltration membranes (Tami, Nyons, France) of molecular weight cutoffs (MWCO) 8 kDa, 3 kDa, and 1 kDa, arranged into two sequential configurations (Figure 1). Every membrane unit operated at volume concentration factor of 1.6, crossflow velocity 3.3 $m \cdot s^{-1}$ and 1 bar of transmembrane pressure. Both permeate and retentate fractions were analysed for their soluble protein content and molecular weight (MW) distribution by Size Exclusion Chromatography. Two *in vitro* activities —DPPH radical scavenging and ferrous chelating activity— were determined on the peptide fractions.

## Results

A protein hydrolysate with degree of hydrolysis 18.5% was obtained after 4-h treatment with Alcalase and 1 h of combined proteolysis with Alcalase and Flavourzyme. The resulting hydrolysate was centrifuged to remove insoluble residues. The supernatant was submitted to membrane fractionation, following two separation sequences as shown in Figure 1. The MW distribution revealed that the final permeates F8F1 and F3F1 presented significant proportion of peptide species within the range 0.3–3 kDa, as well as 16% w/w of free amino acids. Their peptide profiles make them suitable for aquafeed formulation.

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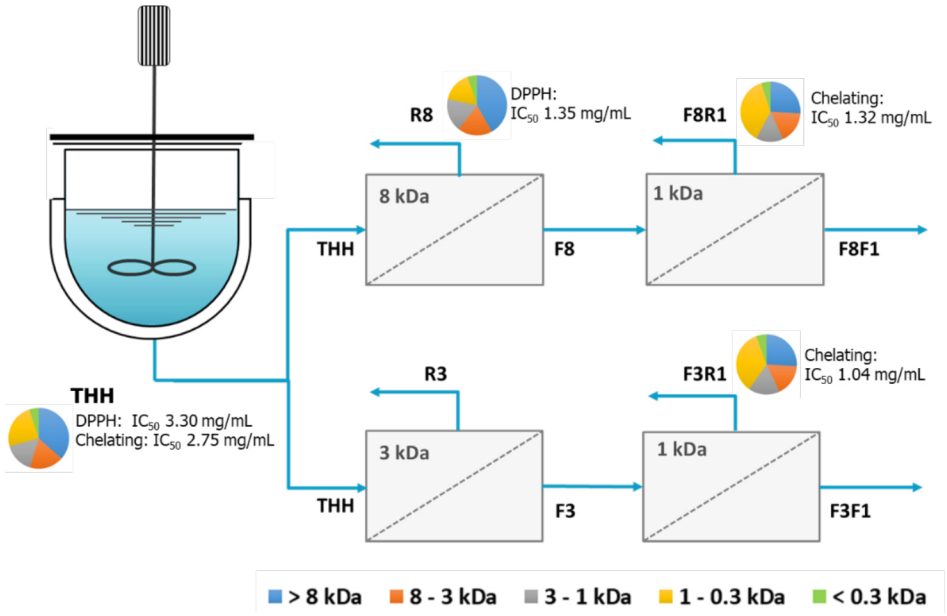


**Figure 1.** Layout of the enzyme hydrolysis and membrane fractionation sequence, showing the MW distribution and *in vitro* antioxidant activities of the peptide fractions.

DPPH radical scavenging and ferrous ion chelation of the tuna head hydrolysate ($IC_{50}$ = 3.30 mg·mL$^{-1}$ and 2.75 mg·mL$^{-1}$, respectively) were significantly improved by membrane fractionation. For instance, metal chelating activity was significantly favoured by the content of low MW peptides, as evidenced by the bioactivity displayed by the final membrane streams F8R1 and F3R1 ($IC_{50}$ = 1.04 and 1.32 mg·mL$^{-1}$, respectively).

## Conclusion

The integrated process proposed in this work allows the valorisation of tuna canning by-products to obtain membrane fractions rich in low MW peptides and free amino acids, as well as displaying antioxidant activities *in vitro*. These fractions could be used as functional ingredients in aquafeed formulation. Future research should focus on their validation through zootechnical experimentation.

## Acknowledgement

This research was funded by the project PCM_00044, granted by the Consejería de Universidad, Investigación e Innovación (Junta de Andalucía), Government of Spain and NextGeneration EU-European Union

# LOOKING FOR OYSTERS FROM THE SKY

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## Introduction

The Pacific oyster, *Magallana gigas*, has established stable populations in over 30 countries, becoming invasive in several of them due to its ecological impact (1). In Spain, specifically in Galicia, it was introduced in the 1980s and has since expanded southward, displacing native species (2). Despite its continued spread, no comprehensive monitoring efforts have been conducted since 2009. This lack of up-to-date data is concerning, especially in light of projected environmental changes that are likely to favor further expansion of this non-native species. Therefore, implementing an effective and continuous monitoring strategy is essential for understanding and managing its distribution. Long-term monitoring of this species is challenging in coastal areas, particularly in intertidal and shallow zones, due to the vast spatial extent, limited accessibility, and high resolution required, among other factors. In this work an approach to address such problems is presented through the use of UAVs (Unmanned Aerial Vehicles) and automated image analysis.

## Material and methods

Ten surveys at representative locations of the central and edge distribution of the Pacific oyster were selected at each of the 9 northern Rías in Galicia (Spain) where *M. gigas* is already naturalized (Ribadeo, Foz, Viveiro, O Barqueiro, Ortigueira, Cedeira, Ferrol, Ares-Betanzos and A Coruña). In each survey, a drone flew over the intertidal zone during the low-tide at a constant altitude of 16 m with a payload composed of an RGB and a multispectral camera to collect images with a maximum resolution of 0,3 cm/pixel. Ground-truth samples were also collected by placing 10-15 quadrats (20 x 20 cm). For each quadrat, GPS coordinates and digital photographs were taken.

High-resolution orthorectified mosaics for the RGB and Multispectral images, as well as Digital Elevation Models (DEMs) were generated with the Agisoft PhotoScan Pro software. Supervised machine learning algorithms were trained based on the annotations made by an expert on the orthomosaics, identifying the objective species. Different deep learning models and techniques were tested to select the one which offers better classification metrics. To assess temporal changes, five of the original sites were re-surveyed one year later, in order to evaluate the algorithm's ability to detect expansion or contraction in oyster coverage over time.




Image 1. Example of oyster segmentation. From left to right: 1. Real image, where green marks indicate correct recognition by the model and red marks indicate false positives. 2. Segmentation mask created by specialists. 3. Segmentation mask generated by the model.

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## Results

The best performing model achieved an accuracy exceeding 90 % for the identification of oysters on both training and test sets created from the original orthomosaics (Image 1). The confusion matrix shows a good trade-off between false positives and false negatives. Furthermore, the comparison of the obtained metrics for different locations and substrates does not show relevant differences, indicating good classification success both in rocky and muddy areas. These classifications were subsequently used to estimate oyster coverage, where we find areas with coverage ranging from 5% to 90% throughout northern Galicia, which matches the expected results after the visual assessment of the locations.

## Discussion

The study combines a series of emerging techniques to complete gaps on population dynamics (AUVs for mapping distribution and numerical modelling), integrate available multidisciplinary information in a common framework (GIS) and deliver a toolbox to assist decision-makers on the management of a non-indigenous species. Results provide new and significant insights to understand the expansion pathways of pacific oysters from a multidisciplinary approach.

This methodology not only evidences the viability of efficient long-term monitoring of *M. gigas*, but also offers a promising tool for tracking other ecologically relevant or conservation-priority species and sets the basis for the implementation of connectivity information and graph theory on the management of sedentary resources, and particularly in the assignment of spatially explicit invasion risk indices for non-native species.

## References

**1.** Abollo et al., 2017. **2.** Des et al., 2022.

# IMPACT OF TREATMENT WITH A SECONDARY BILE ACID (LITHOCHOLIC ACID) ON THE HEPATIC METABOLISM OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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**Introduction**

The growing demand for aquaculture products requires innovative strategies to maximize feed efficiency while maintaining the health and homeostasis of fish. In this context, bile acids (BAs) play a central role in the emulsification and absorption of lipids, in addition to modulating metabolic pathways and physiological responses that remain poorly studied to date. Recently, we demonstrated the impact of secondary bile acids (SBAs) on appetite-regulating mechanisms in rainbow trout (*Oncorhynchus mykiss*) (Pérez-Tierra *et al.*, 2025); however, their role in energy metabolism remains entirely unknown. Therefore, the aim of this study was to investigate the effect of one of the main SBAs—lithocholic acid (LCA)—on the enzymatic activity and mRNA abundance of genes related to key factors controlling both glucose and lipid metabolism, as well as on the levels of key energy metabolites in the liver of rainbow trout.

**Materials and methods**

Juvenile rainbow trout were randomly distributed in five tanks (n=8 fish per tank) and maintained during an acclimation period of two weeks. On the day of the trial, fish were fasted for 24 hours prior to being anesthetized using 2-phenoxyethanol (0.02% v/v). Each fish was then individually weighed and administered an intragastric dose of either distilled water containing 1% dimethylsulfoxide (DMSO; vehicle control) or distilled water with 1% DMSO supplemented with 500 µM lithocholic acid (LCA), at a volume of 1 mL per 100 g body weight. Following treatment, fish were returned to their respective tanks and six hours post-administration, they were again anesthetized and euthanized to obtain liver tissue for quantification of mRNA abundance, levels of metabolites, and enzyme activities.

**Results and discussion**

The relative mRNA levels of key hepatic regulators of glucose and lipid metabolism were differentially affected by LCA administration (Fig. 1A). Regarding glycogen homeostasis, LCA treatment led to an upregulation of glycogen synthase kinase 3 beta (*gsk3b*) mRNAs, which encode an enzyme that phosphorylates and thereby inactivates glycogen synthase, at 6 h post-administration. However, this transcriptional increase did not translate into significant changes in glycogen synthase activity or hepatic glycogen content, suggesting limited functional impact at the time point studied. In relation to gluconeogenesis, LCA significantly reduced the activities of phosphoenolpyruvate carboxykinase (Pepck), fructose 1,6-bisphosphatase (Fbpase), and glucose-6-phosphatase (G6pase) —the three main enzymes constituting the gluconeogenic pathway in the liver (Fig. 1B). This coordinated decrease indicates a lower hepatic gluconeogenic capacity following LCA exposure. To our knowledge, this is the first report describing an effect of SBAs on gluconeogenesis in fish. Interestingly, despite these pronounced reductions in enzyme activity, no significant changes occurred in the mRNA abundance of *pck1, fbp1*, or *g6pc* (Fig. 1A), in contrast to findings reported in mammalian models (Yamagata *et al*., 2004).

In lipid metabolism, the role of BAs in fish has been studied in the context of their emulsifying properties and their contribution to improving lipid absorption. Most available studies have reported a positive impact of dietary bile acid supplementation under high-fat feeding conditions, where hepatic lipid accumulation and metabolic imbalance are common concerns. In this study, treatment with LCA did not result in significant changes in mRNA abundance (Fig.1A) or activity (Data not shown) of major hepatic enzymes involved in lipogenesis or lipolysis, suggesting that under the tested conditions, LCA does not influence hepatic lipid metabolic pathways in rainbow trout.

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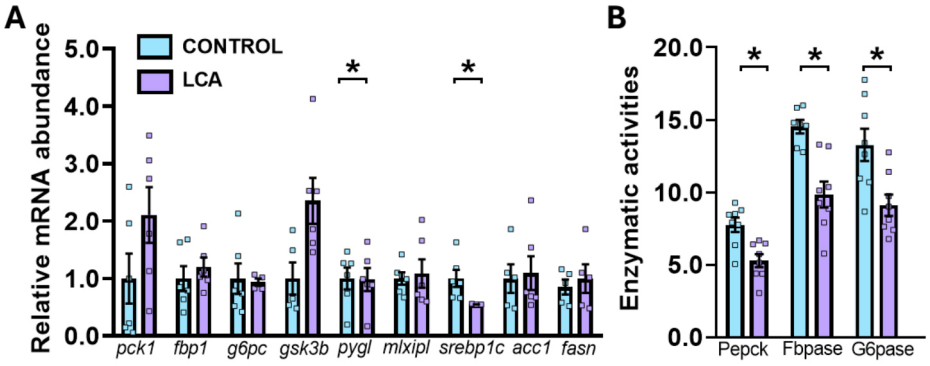


**Figure 1.** (A) Relative mRNA levels of genes encoding key components of hepatic glucose and lipid metabolism and (B) the enzymatic activities (mU·mg-1 protein) of key enzymes regulating gluconeogenesis, were analaysed in rainbow trout 6 hours after intragastric administration of distilled water with 1% DMSO (control) or containing 500 μM lithocholic acid (LCA) at a volume of 1 mL per 100 g body weight. RT-qPCR results were normalized to *actb* (encoding β-actin) and *eef1a1* (encoding elongation factor 1α) mRNA abundance and are presented as fold-change relative to the control group. Data are shown as mean ± SEM (n = 6). Statistical differences between control and LCA-treated group were determined using Student's t-test, with significant differences denoted by * ($p < 0.05$).

## Conclusions

The results of this study demonstrate that intragastrical administration of LCA leads to an apparent short-term inhibition of hepatic gluconeogenesis in rainbow trout, while it does not appear to affect lipid metabolism. Taken together, these findings provide fundamental insight into the short-term impact of SBAs—specifically lithocholic acid—on glucose and lipid metabolism in rainbow trout. This knowledge may serve as a basis for guiding future applications in the development of functional feed formulations for aquaculture.

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# GENOME ANALYSIS OF *Vibrio alginolyticus* AND *Vibrio diabolicus* ISOLATES FROM BIVALVE AQUACULTURE ENVIRONMENT IN THE ADRIATIC SEA

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**Introduction**

Bacteria of the genus Vibrio are ubiquitous in the marine environment. Vibrio species are also known to be opportunistic pathogens for farmed and wild marine organisms, including bivalves. As bivalves are able accumulate microorganisms by filtration, there is a risk of pathogen transmission along the food chain through the consumption of seafood products. Despite their diversity, knowledge about the pathogenic properties and the infection risks from non-cholera *Vibrio* species in the aquaculture of bivalve in the Adriatic Sea is currently very limited.

This aim of the study was to gain a deeper insight into the genome of potentially pathogenic *Vibrio* isolates from the Adriatic bivalve aquaculture environment through high resolution whole-genome sequencing and genome analysis. The study focussed on the presence of virulence factors and antibiotic resistance genes, as well as mobile genetic elements that mediate horizontal gene transfer related to the ability of bacteria to cause disease and survive in different environments. A set of isolates of *Vibrio alginolyticus* and *Vibrio diabolicus* were obtained from samples of farmed Mediterranean mussel *Mytilus galloprovincialis*. An exotic tunicate species *Clavelina oblonga*, which is usually closely associated with growing installations and dominates the biofouling community in the Northern Adriatic [1], was included in the investigation as a potentially relevant reservoir for *Vibrio* species with the capacity to cause infection in aquaculture areas, including the currently uninfected sites [2].

**Materials and methods**

Tissue homogenates of *M. galloprovincialis* and tunicate *C. oblonga* collected from bivalve farms in the northern and southern Adriatic were serially diluted and inoculated onto *Vibrio* selective media (thiosulphate–citrate–bile salts–sucrose agar). Representative overnight grown colonies were screened by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), as previously described [3]. Isolates that showed MALDI-TOF MS identity corresponding to *V. alginolyticus* were selected for confirmation of identity by sequencing of the RNA polymerase sigma factor gene (*rpo*D). A representative set of isolates identified as *V. alginolyticus* and *V. diabolicus* was selected for whole-genome sequencing performed on Illumina NovaSeq X Plus platform, to generate paired-end 150nt reads (Novogene, EU). Raw sequences were processed using online tools for quality checking, read trimming and assembly into contigs.

All sequences were scanned using the Virulence Factor Database - VFDB [4] with the full set of verified and potential virulence factors. The sequences were also screened for antibiotic resistance genes using the Comprehensive Antibiotic Resistance Database - CARD [5] and ResFinder *v* 4.7.2. [6,7]. Mobile genetic elements (MGEs), such as genomic islands, insertion sequence elements and prophages were predicted using IslandViewer 4 [8], ISEscan *v* 1.7.3. [9] and PHAge Search Tool Enhanced Release (PHASTER) [10], respectively.

**Results and discussion**

Our results show that *V. alginolyticus* and *V. diabolicus* from bivalve aquaculture in the Adriatic Sea share many of the genes encoding virulence factors with *Vibrio* species previously isolated from different environmental samples, that play a role in adherence, biofilm formation, evasion of phagocytosis, chemotaxis, motility, acquisition of iron, delivery of bacterial proteins, quorum sensing and haemolytic activity. Genes encoding for resistance to macrolides, fluoroquinolones, penicillin beta-lactams, tetracycline and fosfomycin were also identified. Several genomic islands were detected with genes encoding members of different transposase families, recombinases, phage proteins, lipid metabolism proteins, virulence factors, antibiotic resistance components and many hypothetical proteins, were detected in all isolates. A considerable number of insertion sequences and repeated regions as well as intact prophages were also found. These particular genetic features indicate the ability of bacteria to acquire new genetic information through horizontal gene transfer, which contributes to bacterial evolution, survival and pathogenesis. Overall, these results highlight the risk associated with the spread of bacteria in aquaculture which could have an impact on the profitability of production and the safety of public health.

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**Acknowledgements**
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# INFLUENCE OF PLOIDY AND GROWTH PHENOTYPE ON THERMAL STRESS RESPONSES IN *Mytilus edulis*

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Mussels (*Mytilus edulis*) can show wide growth variability, even among same-aged individuals reared under similar conditions. Triploidy can influence growth rates as energy can be diverted from reproduction to somatic growth. Furthermore, the response of triploid mussels to heat stress remains poorly understood. This study investigated how growth phenotype (i.e., slow versus fast growers) and ploidy (i.e., diploids versus triploids) influence the response of mussels to thermal stress. Within each ploidy group, slow and fast growers (bottom and top 20% by size) were characterized across three mussel families. Each family was tested separately, where mussels were placed in individual respirometry chambers and exposed to thermal stress starting at an acclimation temperature of 18°C. The temperature was increased by 2°C every three days until it reached a sub-lethal temperature of 26°C. Shell lengths were measured at the start and end of the experiment, while feeding rates were assessed at 18, 22, and 26°C, and respiration rates were measured continually every 2h. Throughout the experiment, samples were collected for genotyping, RNA-sequencing, metabolomics, gill histology, and flow cytometry. This study aims to better understand how growth phenotype and ploidy influence mussel responses to thermal stress, with potential applications in mussel aquaculture through the selection of resilient growth lines.

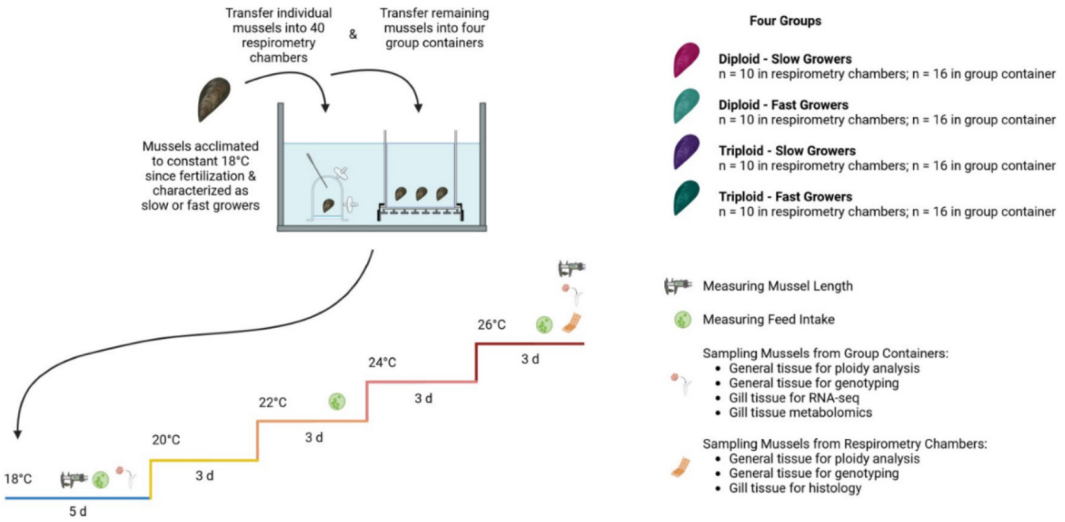


Figure 1. Experimental design of the temperature ramp (from 18 to 26°C) for slow and fast growers from both diploid and triploid mussels across 3 families, with morphological and tissue sampling denoted.

# BREEDING CLIMATE-READY BIVALVES: UNRAVELING THE GENOMIC BASIS OF HEATWAVE TOLERANCE IN *Ruditapes philippinarum*

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Bivalve aquaculture is an important activity able to provide healthy food with a low carbon footprint. This activity is threatened by climate extreme events such as marine heatwaves (MHWs), which are increasing their frequency, duration and intensity every year. In shellfish farming the scope for contrasting the impact of MHWs is limited. Selective breeding for organisms that are more resilient to MHW may be an effective solution against these stressful events and may, in turn, sustain the clam aquaculture sector. Within this framework, genome wide association studies (GWAS) supported by SNP array genotyping is a powerful tool to investigate the genetic basis of such complex (and possibly multigenic) traits.

For this task we initially designed a novel multi-species Axiom SNP array (60K) with ~45K SNPs for the Manila clam (*Ruditapes philippinarum*) and ~15K SNPs for the carpet shell clam (*R. decussatus*). The array was subsequently used aiming to identify quantitative trait loci (QTLs) significantly linked with the trait resistance to MHWs.

To fulfil our aim, we exposed a population of 1000 Manila clams, obtained from a mass spawning event at the Satmar hatchery, to a simulated MHW that mimicked a natural event which occurred in the Venice lagoon in 2015, with water temperatures fluctuating daily between 31 and 35 ˚ C. Animals were randomly split across 10 aquaria and the MHW was replicated across all the experimental tanks, lasting 15 days. After this period, we stopped the MHW and kept the remaining animals at 25 ˚ C until no more casualties were observed in the population. All samples were genotyped with the Axiom SNP-array. We evaluated the resistance to MHW as both a binary trait (i.e. resistant vs susceptible) and as a continuous trait (i.e. time to death). By identifying genetic markers associated with MHW resistance, this research paves the way for developing more resilient clam populations through genomic driven selective breeding, potentially revolutionizing the aquaculture industry's ability to adapt to climate change challenges.

# MARINE PROBIOTICS WATER SUPPLEMENTATION EFFECTS ON *Litopenaeus vannamei* AND ITS POSITIVE IMPACT ON SURVIVAL RATE AND MICROBIOTA AFTER AN AHPND STRESS

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## Introduction

The aquaculture industry is continuously interested in innovative practices with the aim of improving farm practices while ensuring sustainable development. This is reflected in a global demand for shrimp quality and a minimal impact on the environment. One of the key strategic priorities is to improve resistance to pathogenic bacteria causing outbreaks and economic losses, and probiotics offer a potential strategy to achieve this goal. Probiotics are defined as microorganisms exerting a beneficial effect by modulating microbiota when they are ingested in adequate quantity. They are good candidate to improve shrimp immune resistance, to reduce the use of antibiotic and lead to a more sustainable aquaculture. In this study, we used probiotic strains which are native from the marine environment and are well adapted to the aquatic environment.

## Material and methods

A 7-week water supplementation with or without marine probiotics was performed in 800-liter ponds on *Litopenaeus vannamei* PL12 in triplicate (n = 1000, water temperature = 28°C). This supplementation was continued during a 15-day challenge test with *Vibrio parahaemolyticus* causing AHPND in 40L ponds (n = 60, 3 replicates, water temperature = 28°C). The capacity of the marine bacteria consortium on zootechnical parameters and shrimp survival rate was evaluated. A metabarcoding analysis was done on dead and alive shrimps to understand mechanisms involved at the microbiota level. Previously, an *in vitro* study was realized to understand the antibiofilm and antibacterial potential of one of these marine probiotic strains on pathogenic *V. harveyi* ORM4. Three different biofilms were realized by testing the activity of the probiotic supernatant as a curative or preventive solution and with a co-inoculation with the pathogen. Liquid chromatography coupled with a mass spectrophotometer was also performed on probiotic supernatant to investigate the production of antibiofilm compounds (Petit et al., 2024).

## Results

The results demonstrated a survival rate multiplied by 2.7 at the end of the challenge compared to the control group. The metabarcoding results showed that marine probiotics, added to the water, had a positive impact on the shrimp larvae gut. Indeed, the microbiota was marked by the presence of the *Pseudoruegeria* genus, known to be a defense mechanism against pathogens. This genus, coupled with marine probiotics, allowed shrimp larvae to have the therapeutic arsenal to fight the pathogen, explaining the better survival rate. The results show the ability of marine probiotics to influence the shrimp gut microbiota. Another characteristic of marine probiotics is their properties to have antibacterial and antibiofilm activity against pathogenic *V. harveyi* ORM4 when the probiotic is co-inoculated with the pathogen or used as a preventive or curative solution. We demonstrated in parallel the ability of this strain to synthesize lipopeptides which presented an antibiofilm activity against *V. harveyi* ORM4.

## Conclusion

These studies demonstrated the ability of marine probiotics to limit the development of pathogens thanks to their antibacterial and antibiofilm activity but also to help shrimp larvae fight against stress caused by *V. parahaemolyticus* by modulating the microbiota and improving the survival rate.

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# CRAYFISH FOR SLUDGE MANAGEMENT IN AN AQUAPONIC SYSTEM

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## Introduction

Aquaponics is a sustainable aquaculture method that combines fish farming and plant cultivation in a single closed recirculating system (FAO, 2019). Despite this being a well-acknowledged circular economy approach in RAS, the method is still under intensive development to increase its efficiency and sustainability. One common problem in aquaponic systems remains the accumulation of organic sludge, which can compromise water quality, lower system performance, and increase maintenance needs (Lenz et al., 2021). An integrated multi-trophic aquaculture approach by adding some filter feeders or detritivores to the standard aquaponics can help manage solid wastes and increase system productivity. In this study, we investigated whether incorporating detritivorous crustaceans could help reduce sludge production and improve system efficiency, compared to a standard aquaponic setup.

## Material and methods

Experimental cultivation of rainbow trout (*Oncorhynchus mykiss*), lettuce (*Lactuca sativa*), and noble crayfish (*Astacus astacus*) was performed in a small aquaponics system (a recirculating aquaculture system connected to three growth beds for plants) at Klaipeda University Marine Research Institute Fisheries and Aquaculture Laboratory. Three production cycles of 28-40 days were carried out. For 20 kg of rainbow trout biomass, 15-20 crayfish were added.

During each cycle, sludge production, water quality, and the growth of fish, crayfish and lettuce were monitored. Each cycle started with 19-20 three-week-old lettuce seedlings planted into the growth beds with clay pebbles. The average individual plant weight and total biomass were estimated. For fish and crayfish, biomass produced, average weight gain, feed consumption rate (FCR) and survival rate (%) were counted.

Sludge from the fish and crayfish tanks was collected once a week using a mesh bag and weighed. The total amount of sludge from the fish and crayfish tanks, the amount of sludge consumed by crayfish (g $h^{-1}$), and the efficiency (%) of sludge removal by crayfish were calculated. This study demonstrated that integrating crayfish into an aquaponics system maintained optimal water quality and had no negative impact on fish or lettuce growth.

## Results

Sludge collection revealed that crayfish tanks generated nearly half the sludge amount compared to fish tanks (82 g and 47 g, respectively). Crayfish actively consumed some sludge (on average, crayfish remove 3,5 ± 11,28 g of sludge per hour from the fish tank); however, the overall sludge removal efficiency was low.

## Conclusion

The study concludes that while crayfish can contribute to sludge reduction in aquaponic systems, optimizing crayfish density, feeding regimes, and tank design is essential for improving sludge removal efficiency and enhancing system sustainability. An integrated multi-trophic approach will be further tested with other crustacean species.

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# ASSESSMENT OF INTERACTIONS BETWEEN SUSTAINABILITY AND WELFARE ISSUES IN VIETNAMESE SHRIMP FARMS

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## Introduction

Current researchers offer some analysis of welfare trade-offs with individual sustainability pillars, or sustainability pillar(s) and welfare; but rarely consider sustainability and welfare together. The challenges in aquaculture are, for example, while welfare improvements, they often conflict with environmental sustainability goals like resource conservation or effluent reduction; while prioritizing environmental objectives can sometimes undermine welfare outcomes, although welfare and environmental sustainability are closely aligned goals (Albalat et al. 2022). This research investigated the interactions among sustainability pillars and welfare management across various shrimp farming intensities, from extensive to super-intensive systems.

## Materials and methods

Our work are conducted in the four main shrimp farming Mekong-delta Vietnamese provinces (Ca Mau, Bac Lieu, Soc Trang and Kien Giang, see Fig. 1). The surveys included 210 farmers who provided responses about their farms, with corresponding answers about their demographic and shrimp farm's details in the year 2024.

To analyze the data, we utilized Principal Component Analysis to develop composite indices for sustainability pillars and welfare, with each index standardized to range from 0 to 100. Structural Equation Modeling was then employed to explore the interactions between sustainability and welfare across varying shrimp farming intensities. Accordingly, we follow SEM with the inner model as below:

| | Hypothesis | Equation |
|---|---|---|
| $EiI = \beta_1 WI + \beta_2 EnI + \beta_3 SI + \in_1$ | (H1, H2, H3) | (Eq. 1) |
| $WI = \beta_4 EnI + \in_2$ | (H4) | (Eq. 2) |

where , are the path coefficients representing estimating the strength of each relationship and is the error term. The relationships are linear regressions within the structural model. In Eq.1, economic and welfare relationship by (H1) examinate how shrimp welfare affects economic efficiency. Besides, the model from Eq.1 also highlights other key relationships: the environment and economic relationship by (H2), and social influence economic performance by (H3). Additionally, the model from Eq. 2 assesses the interaction between shrimp welfare and environment sustainability relationship by (H4).

## Results

Fig. 1 illustrates the mean values (range 0 - 100) of the sustainability (economic, environmental, social), and welfare management indexes across three shrimp farming systems. Overall, intensive farms require higher input costs and rely less on natural ecological processes while increasing their dependence on intensive feed and aeration, leading to the lowest economic index (Ngoc et al. 2023). The environmental index is also less emphasized in intensive systems, as they focus more on maintaining pond water quality rather than promoting ecological balance. The shrimp welfare management index, however, is not much varied across all farming types, as econ-environmental priorities often take precedence over direct welfare investments.

Table 1 identified several important patterns in the interactions. In super-intensive and intensive farms, we observed a strong negative relationship between welfare improvements and economic performance, indicating that prioritizing welfare measures often incurs a financial trade-off. Across all farm types, environmental improvements were found to significantly reduce economic outcomes, with the most pronounced negative effect occurring in intensive farming systems. However, environmental enhancements were shown to significantly improve welfare outcomes across all shrimp farms, with the strongest positive impact observed in extensive and integrated systems.

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Fig 1. Sustainable pillars and welfare indexes across shrimp farming systems

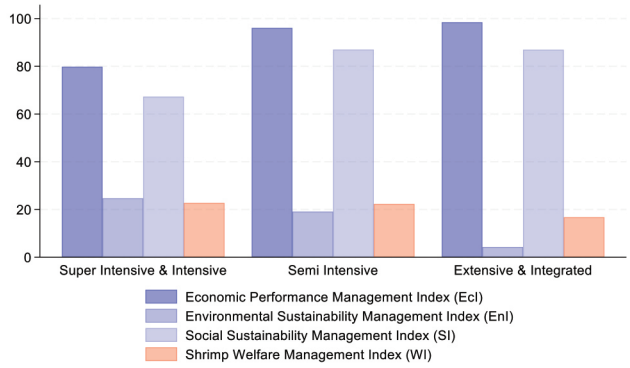


Table 1. The relationship between shrimp sustainability and welfare – SEM modeling

| | | All farms | Super intensive and intensive | Semi intensive | Extensive and integrated system |
|---|---|---|---|---|---|
| WI to EcI | Coeff | -0.0633 | -1.220* | -0.00781 | 0.0145 |
| | S.E | (0.0600) | (0.720) | (0.0228) | (0.0131) |
| EnI to EcI | Coeff | -0.262*** | -0.402*** | -0.0131 | -0.0459** |
| | S.E | (0.0845) | (0.117) | (0.0137) | (0.0198) |
| SI to EcI | Coeff | 0.105*** | -0.0201 | 0.0282 | 0.00580 |
| | S.E | (0.0386) | (0.100) | (0.0256) | (0.0105) |
| EnI to WI | Coeff | 0.131*** | 0.0809 | -0.000555 | 0.203** |
| | S.E | (0.0419) | (0.0526) | (0.0481) | (0.0823) |
| Observation | | 210 | 36 | 46 | 128 |

Note: ***p < 0.01, **p < 0.05, *p < 0.1.

**Discussion and Conclusion**

Extensive farming systems are often characterized by lower stocking densities and more natural rearing conditions, enabling shrimp to thrive in a more ecologically balanced environment. In contrast, intensive and semi-intensive systems typically prioritize productivity, which can increase disease susceptibility and stress for shrimp. As stocking densities rise, shrimp have less space to move, leading to reduced oxygen levels, higher waste accumulation, and greater pathogen transmission. These conditions ultimately increase the risk of shrimp to experiencing poor welfare if the systems are not well managed.

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# SINGLE CELL TRANSCRIPTIONAL PROFILING OF GILTHEAD SEABREAM (*Sparus aurata*) GILLS. A USEFUL TOOL FOR MONITORING GILL HEALTH IN AQUACULTURE

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## Introduction

Teleost gill is a complex multifunctional organ essential for several physiological functions, such as gaseous exchange, osmoregulation, excretion of nitrogenous waste, pH regulation, immunity, and hormone production. Gill health is a key aspect in aquaculture and, due to the direct contact with the environment, it can be affected by different factors such as pathogens or environmental stressors.

Our current knowledge on the gill transcriptional responses in gilthead seabream is based on bulk transcriptomics of whole tissue, making it difficult, if not impossible, to link a response to a specific cell type or structure. In this work, we performed a single cell RNA-sequencing study to obtain a transcriptional atlas at single cell resolution that allowed us to define different cell types and subtypes in the healthy gill and find marker genes that can be used for monitoring gill health in aquaculture.

## Material and Methods

Two healthy gilthead seabream of 100 g were euthanized and bled completely to avoid erythrocyte contamination. Left gill arches were extracted and single cell suspension of gill filaments were obtained using a protocol based on incubation with EDTA, DTT, DNaseI and collagenase, and mechanical dissociation. Single cell suspensions were fixed with the Parse Biosciences Fixation Kit and 8,300 fixed cells were subjected to combinatorial barcoding and library preparation using the Evercode WT v3 kit.

After Illumina sequencing, quality filtering and data analysis was performed using Trailmaker. Mapping was performed using fSpaAur1.2 genome assembly from NCBI. Seurat was used for clustering and differential expression analysis. Marker genes for each cell cluster were used to perform gene ontology (GO) analyses and identify cell types.

## Results

After quality filtering, 3,000 cells per sample, with a median of 550 genes per cell were obtained. Cells were clustered into 15 groups that were broadly annotated by the differential expression of marker genes and enriched GO terms (Fig. 1a).

The largest clusters corresponded to epithelial, vascular and goblet cells, the latest with high expression of mucin 5ac and mucin 2 (Fig. 1b), and markers involved in epithelial cell differentiation and mucus layer. Epithelial cells expressed markers involved in cell junction and adhesion, desmosome or extracellular matrix organization (e.g. collagens, claudins or plakophilins). Transcriptional markers in the vascular cell cluster belonged to GO terms like collagen binding, cell adhesion, circulatory system, etc. Further subclustering of the vascular cell cluster allowed to identify the pavement cell population.

The expression of markers like *chga* or *notch1* allowed the classification of endocrine and neuroepithelial cells, respectively. Endothelial cells were enriched in markers involved in GO pathways related to the establishment of the endothelial barrier, such as *ppp1r16b*, *tjp2*, *krt19* and *cdh5*. A clearly separated cluster, with marker genes enriched for GO terms like ion transport was identified as ionocytes or mitochondrion-rich cells (MRCs), important cells in maintaining osmotic, ionic, and acid-base levels.

Regarding the immune system, three clusters were identified as myeloid cells by the expression, among others, of the cell markers *cd74* and *cd48* (Fig. 1c), and then further labeled as dendritic cells, macrophages and granulocytes. The lymphocyte population contained markers for both, B and T lymphocytes, such as *trbc*, *trac*, *cd40* and *pax5*.

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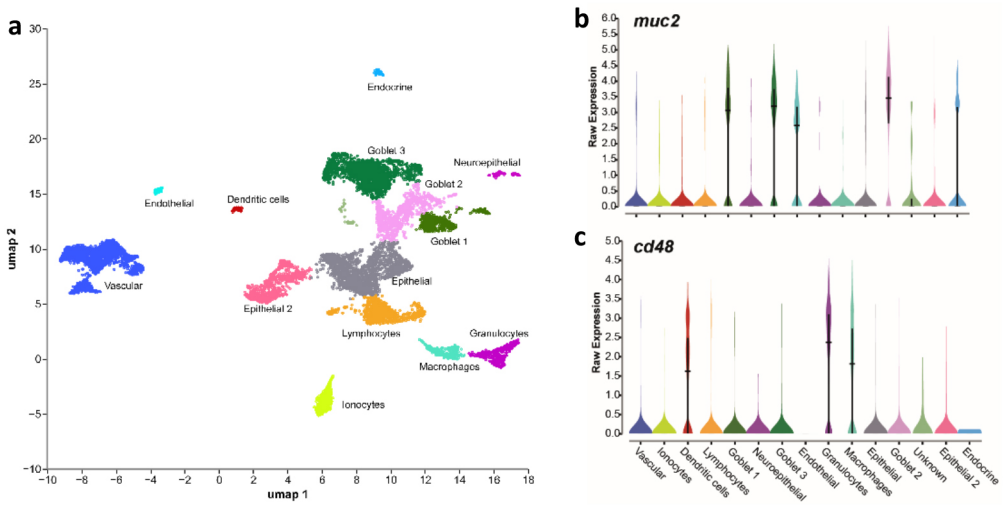


Figure 1: a) UMAP plot of pooled cell data. The plot shows the transcriptional distribution of 15 separate cell clusters. b, c) Violin plots showing the expression level of the goblet cell marker *mucin 2* (b) and the myeloid marker *cd48* (c).

**Conclusions**

This work provides the first transcriptional atlas at single cell resolution of the healthy gilthead seabream gill. We had broadly described 15 different populations based on marker gene expression and GO analysis. Further subclustering of the largest populations will provide markers and the transcriptional profiles for cell subtypes.

This dataset sets the baseline expression in healthy gills and provides a set of markers that will ease the interpretation of future studies aimed to determine gill status under different situations, pathogens or stressors.

**Funding**

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# *IN SILICO* AND *IN VITRO* INVESTIGATIONS OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVA MEAL BIOACTIVITY: MYOTROPIC POTENTIAL ON THE INTESTINAL TRACT OF RAINBOW TROUT (*Oncorhynchus mykiss*) AND ANTIBACTERIAL PROPERTIES AGAINST VARIOUS PATHOGENS

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## Introduction

The industrialization of the aquaculture sector has been accompanied by a strong demand for raw materials to meet growing feed requirements. Fishmeal remains the main source of animal protein despite its high price and harmful impact on marine ecosystems. In this context, a substantial part of the fishmeal in aquaculture feeds has already been substituted by plant-based resources such as soy-based proteins. However, these alternatives also come with their sustainability issues and high environmental impact. Insect-based protein has more recently been proposed as a robust and more sustainable option, with a nutritional profile well adapted to aquatic species. The protein-rich larvae of the black soldier fly (*Hermetia illucens*) (BSF) are currently farmed industrially to produce meal. This study aims to assess the bioactive potential of the soluble fraction of insect meal – water soluble fraction (WSF) – potentially correlated with health and well-being claims.

## Materials and methods

Total RNA from *Hermetia illucens* larvae was extracted to construct a transcriptome. Sequencing was performed on an Ilumina NovaSeq 6000 sequencer using a cDNA library. Sequence cleaning and transcriptome assembly and annotation were carried out by the ABiMS platform (Roscoff biological station - CNRS). *In silico* analysis of the transcriptome was carried out using PepTraq software to identify the myotropic neuropeptides and antibacterial peptides expressed by BSF larvae. At the same time, peptides extracted from water soluble fraction (WSF) were used for functional tests. Myotropic activity tests were carried out on the proximal part of the intestine of rainbow trout (*Oncorhynchus mykiss*) using an electrophysiology system to measure intestinal contraction. Antibacterial activity tests were carried out through the growth of a wide range of pathogens of aquatic organisms – *Aeromonas salmonicida, Flavobacterium psychrophilum, Yersinia ruckeiri, Lactococcus garvieae, Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio splendidus, Vibrio anguillarum, Vibrio vulnificus, Vibrio harveyi, Vibrio fisheri, Vibrio penaeicida* – was assessed. Growth inhibition percentages were determined in triplicate by liquid growth inhibition tests using a Bioscreen system.

## Results

The *Hermetia illucens* larva transcriptome included 355,085 transcripts, 69,323 (19.5%) of which possessed a signal peptide. Twenty-six protein precursors corresponding to 22 families of neuropeptides with potential myotropic activity were identified. Seventy-two protein precursors of antibacterials grouped into 4 families were also identified: defensins (30), cecropins (27), attacins (3) and diptericins (12).

Functional tests of myotropic activity revealed that the amplitude and tone of contractions were proportional to the WSF concentration (Figure 1), highlighting a dose effect and a dose-dependent response.

Antibacterial activity measurements revealed three types of effect on the growth kinetics of the pathogens tested in the presence of crude WSF extracts (Figure 2): i) reduced growth, ii) a bacteriostatic effect, and iii) a bactericidal effect. Growth inhibition was observed on *Lactococcus gaerviae* and *Vibrio anguillarum*. A bacteriostatic effect was observed on *Flavobacterium psychrophilum*, *Vibrio fisheri,* and *Vibrio splendidus*. Finally, a bactericidal effect was observed on *Vibrio penaeicida* whatever the crude extract extraction method, which turned into a bacteriostatic effect from the dilution corresponding to 80 $OD_{214}$ units.

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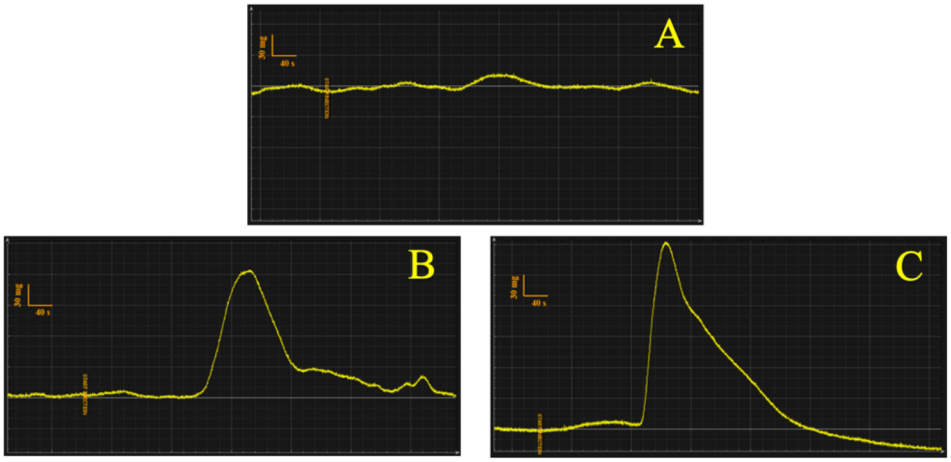


*Figure 1: Measurement of myotropic activity induced by the WSF extract at different concentrations resulting in contraction of the proximal intestine of Oncorhyncus mykiss. Dose effect: perfusion of an extract concentrated to 1 $OD_{214}$ unit (A), 40 $OD_{214}$ units (B), and injection of the crude solution (C).*

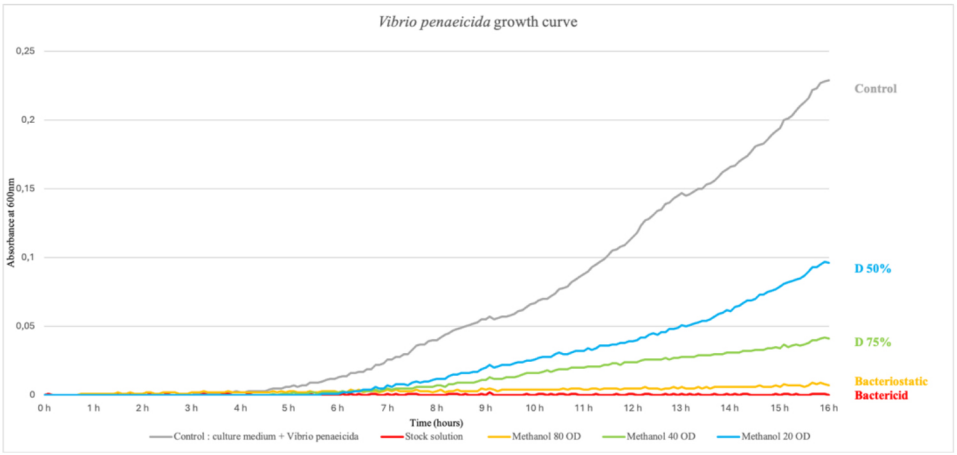


*Figure 3: Vibrio penaeicida growth curve: type of effect observed as a function of the WSF extract concentration (i, ii, iii)*

## Conclusion

This initial *in vitro* approach confirmed the bioactive potential of BSF meal. Its myotropic effects could lead to improved feed digestibility, and its antibacterial properties could significantly reduce the use of antibiotics at a time when antibiotic resistance is a growing concern.

# EFFECTS OF BLACK SOLDIER FLY (*Hermetia illucens*) PROTEIN EXTRACTS ON GROWTH PERFORMANCE AND INTESTINAL TRANSCRIPTOME IN RAINBOW TROUT (*Oncorhynchus mykiss*) UNDER FISHMEAL REPLACEMENT CONDITIONS

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**Introduction**

Climate change and geopolitical instability are contributing to growing uncertainty regarding the availability of raw materials for animal feed, particularly protein sources used in aquaculture. This underscores the importance of continued research into alternative protein ingredients. In recent years, considerable attention has focused on assessing the nutritional value of insect-based products for farmed fish, especially carnivorous species such as salmonids (1). However, with ongoing advancements in the industrial processing of insects into protein meals, further evaluation is needed to determine the nutritional quality of these next-generation ingredients. This study aimed to assess the impact of partially and fully replacing fishmeal-derived proteins with insect-derived proteins in diets formulated for rainbow trout (Oncorhynchus mykiss). The insect proteins were sourced from three by-products of black soldier fly (BSF) processing: a crude insect meal (CIM), a refined insect meal (RIM) with reduced chitin content, and a protein extract derived from the aqueous fraction separated during the wet processing of BSF larvae into protein meal and oil, and commonly referred to as stickwater (SW). Due to its high content of water-soluble compounds, stickwater is particularly rich in low molecular weight proteins, peptides (including bioactive peptides), and micronutrients, notably minerals.

**Materials and methods**

A digestibility trial and a 12-week growth experiment were conducted on juvenile rainbow trout to evaluate seven experimental diets. The control diet included 17% fishmeal (FM). Two diets involved complete replacement of fishmeal protein with either crude (CIM100) or refined (RIM100) insect meal. Four additional diets replaced 50% of fishmeal protein with CIM (CIM50), RIM (RIM50), or a blend of 70% CIM or RIM and 30% stickwater (CIM-SW, RIM-SW). Diets, faeces, and whole fish were analyzed for proximate composition to calculate apparent digestibility coefficients (ADCs) and assess nutrient retention. Transcriptomic analysis of the distal intestine was performed to evaluate molecular responses to dietary treatments. Data were analyzed using a two-step approach: a two-way ANOVA assessed the effects of insect meal type and substitution level among the six insect-based diets, followed by a one-way ANOVA comparing all diets, including the control.

**Results**

Partial or total replacement of fishmeal with crude (CIM) or refined (RIM) insect meal reduced the apparent digestibility coefficients (ADCs) for protein, lipid, and energy compared to the fishmeal (FM) control, with the lowest values observed in the CIM-100 diet. However, supplementing 50% replacement diets with stickwater (SW) restored digestibility to control levels. Growth performance was not significantly affected by diet. Absolute feed intake increased with SW inclusion, and, when normalized to metabolic body weight, was also higher in CIM-50 and RIM-100 diets. Feed efficiency declined with full fishmeal replacement by CIM compared to control FM diet. Insect-based diets increased digestible nitrogen, fat, and energy intake, especially with SW. Fecal nutrient losses were also higher, particularly under full replacement, but were significantly reduced by SW in the 50% substitution diets. Nitrogen, fat, and energy gains and retentions were not significantly different among diets. Transcriptomic analysis of the distal intestine revealed limited changes between 50% and 100% substitution levels (2–3 differentially expressed genes, DEGs, per insect meal type). Comparing RIM-based diets revealed 27 DEGs, versus only 2 in CIM-based diets. Relative to the FM control, RIM diets showed 27 DEGs, and CIM diets 7, many associated with immune and inflammatory responses. Ladderlectin a and b were upregulated with insect diets, while samd9, an antiviral gene, was downregulated in CIM-100-fed fish.

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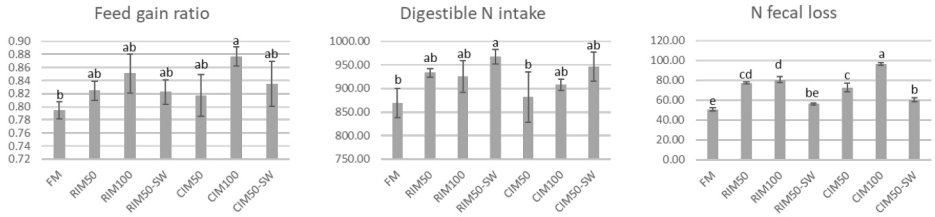


**Discussion**

Diets with partial or total replacement of fishmeal by insect proteins of varying quality supported growth performance comparable to that of the control diet containing 17% fishmeal. Chitin content did not appear to limit growth, but feed efficiency declined with insect-based diets. These insect-based diets led to higher nutrient intake and fecal losses, resulting in similar nutrient gains and retention. This potentially could reduced economic and environmental sustainability, particularly with crude insect meal, which showed the poorest efficiency when used in full replacement. Although stickwater improved digestibility in insect-based diets, its growth-promoting effect, as observed with stickwater issued from fishmeal production, in salmon (2), was less evident here. This may be attributed to the less balanced amino acid profile of insect meal compared to fishmeal. Overall, refined insect meal exerted a more pronounced effect on the intestinal transcriptome than crude insect meal. Notably, *ladderlectin a* and *b*, key components of fish innate immunity involved in defense against bacterial infections (3), were upregulated in trout fed insect-based proteins. This response may reflect the ability of ladderlectins, which possess sugar-binding domains, to recognize and bind chitin, a long-chain polymer of N-acetylglucosamine present in insect meals. In contrast, samd9, a gene linked to antiviral defense and potentially acting as a pattern-recognition receptor for microbial double-stranded DNA or RNA (4), was downregulated in trout fed the diet with full crude insect meal replacement. These immune-related transcriptomic shifts highlight the need for pathogen challenge trials to assess the health robustness of trout fed insect-based diets.

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# ASSESSING HOW TRANSPORT INDUCED ACUTE STRESS IN RAINBOW TROUT *Oncorhynchus mykiss* JUVENILES MAY REVEAL POTENTIAL WELFARE BIOMARKERS

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**Introduction**
Improving the welfare of fish in aquaculture is an essential step towards creating a healthier and more sustainable industry. Healthy fish are more resilient to disease, which means less reliance on chemical treatments. The effect of stress on the immune system has been widely investigated and chronic stress is known to suppress or dysregulate immune function and increase susceptibility to disease. However, more efforts need to address the effects of acute stress in fish, which seem to have both stimulatory and inhibitory actions on immune functions. This study focuses on how acute stress, specifically from transportation, affects juvenile rainbow trout (*Oncorhynchus mykiss*), with particular focus on its impact on the immune system, the fish's ability to recover from these challenges and, by analyzing gene expression, attempt to identify potential welfare biomarkers.

**Material and Methods**
A trial was devised to evaluate the stress response in rainbow trout juveniles after transport. Fish (approx. 18 g) were randomly distributed into 9 tanks, with 12 individuals per tank and hand fed to satiation 2 times a day with a commercial diet. Following an acclimatization period, fish from three tanks (n=3) were sampled to serve as a non-stressed control. The fish of each remaining tanks were transferred, to a plastic container (≈25 kg/m$^3$) with an $O_2$ saturated atmosphere and transported for 2 h. Upon arrival, 3 groups of transported fish were immediately sampled. The others were accommodated in the original system and sampled 24 h post-arrival. Plasma was used for cortisol, metabolites and humoral immune parameters. Liver was sampled for the analysis of metabolites and oxidative stress responses. Brain and head kidney were collected for gene expression analysis.

**Results**
Results showed a significant increase in plasma cortisol and glucose levels with a simultaneous decrease of hepatic glycogen and glucose immediately after transport for 2h. Plasma immune parameters showed a significant increase of nitric oxide levels immediately after stress-induced transport. Regarding metabolites, triglycerides increased in plasma whereas lactate decreased in the liver 24 h after stress. In regard to gene expression in the brain an increase in the level of mRNA expression of *crhbp* immediately after transport was observed, as well as its decrease 24h after stress. A similar pattern was observed in the levels of gene expression in the head-kidney, in relation to the genes *hsp70*, *il10* and *arg2*.

**Discussion**
Preliminary results suggested a stress induced response with a primary and secondary response pattern, specifically an increase in plasma cortisol and the rapid release of energy from hepatic carbohydrate reserves. An increase in plasma triglycerides during recovery might suggest a shift in energy use, with the fish relying more on fats as a longer-term energy source. The increase of level of expression of *crhbp* in the brain and *hsp70*, *il 10* and *arg2* immediately after stress, and their subsequent decrease 24 h after stress indicate a well-regulated response of fish to the transport stress, that successfully manage the cellular and inflammatory challenges, and that by 24 hours, had largely returned to a state of physiological homeostasis.

In conclusion, besides plasma cortisol, the expression of genes such as brain *crhbp*, and head-kidney *hsp70*, *il10* and *arg2* seem to be good markers of both stress-induced transport episodes and following its recovery.

**Acknowlegments**
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# EFFECT OF THE USE OF A SETTLING CHAMBER IN THE CULTIVATION OF *Penaeus vannamei* AND *Salicornia neei* IN BIOFLOC-BASED AQUAPONICS

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## Introduction

Although freshwater aquaponics is the most practiced and studied, integrating the cultivation with halophyte plants (salt-tolerant) can be an alternative to the limited use of water for food production and soil salinization. Species such as *Salicornia neei*—commonly known as salicornia or sea asparagus—are of great interest in the global market for gourmet cuisine. The design of aquaponic systems often varies based on production objectives and the species being cultivated. To optimize performance, it is essential to understand the dynamics of solids production and manage nutrient-rich wastewater effectively, ensuring its efficient reuse for plant growth. Given the importance of managing residual nutrients and protecting plant roots from possible negative effects caused by suspended solids, this study aimed to evaluate the use of a settling chamber in a biofloc-based aquaponic system for the production of marine shrimp *Penaeus vannamei* and the halophyte *Salicornia neei*, and to assess its effects on sludge production.

## Material and methods

The experiment was conducted over a 54-day period, at the Marine Shrimp Laboratory (LCM) of the Federal University of Santa Catarina, southern Brazil.

To evaluate the effects of using a settling chamber in the aquaponic system, two treatments were tested: one with settling and one without settling. Each aquaponic experimental unit comprised a circular polyethylene tank (800 L useful volume) for shrimp cultivation and a hydroponic bench for plant growth. The shrimp tanks were equipped with a heater, aeration hose, and four vertical artificial substrates. In the treatment with settling, a cylindrical fiberglass settling chamber with a conical bottom (90 L capacity) was attached.

The aquaponics structure was constructed based on the design by Pinheiro et al. (2017), with modifications. The hydroponic bench consisted of four PVC pipes arranged side by side on wooden supports. Each bench had 0.33 m² of planting area, with 28 seedlings of *S. neei* per experimental unit. In the treatment without settling, water was continuously pumped from the shrimp tank to the hydroponic bench at a flow rate of 3.0 L $min^{-1}$ using a submerged pump and returned to the tank by gravity. In the treatment with settling, water was pumped continuously to the center of the settling chamber, allowing solids to settle along the chamber's conical bottom. Overflow water was then distributed into each irrigation channel and returned to the shrimp tank by gravity. To maintain the proper concentration of suspended solids, every thirty minutes the solids accumulated in the settling chamber were pumped back to the tank for 40 seconds using an electric pump connected to the bottom outlet of the settling chamber, at a flow rate of 15 L $min^{-1}$.

One day before the beginning of the experiment, the tanks and settling chambers were filled with biofloc water from a shrimp nursery tank. Each tank was stocked with 300 shrimp (average weight of 1.20 ± 0.08 g), representing a stocking density of 375 shrimp $m^{-3}$.

## Results and discussion

During the 54-day trial, the reduction of suspended solids in the treatment with settling led to an increase in TAN and $NO_2$ levels, while the concentration of $NO_3$ remained stable. Although water quality parameters were more stable in the treatment without settling, no significant differences were observed between the treatments regarding plant and shrimp production indices (Table 1).

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**Table 1.** Production indices of *Penaeus vannamei* and *Salicornia neei* cultivated in an aquaponic system with and without the use of a settling chamber for 54 days. Data are presented as a mean ± standard deviation.

| Parameters | Treatment | | *p*-value |
|---|---|---|---|
| | With settling | Without settling | |
| *Performance of* P. vannamei | | | |
| Survival (%) | 88.7 ± 6.7 | 86.8 ± 5.1 | 0.6745 |
| Mean final weight (g) | 10.2 ± 0.3 | 10.1 ± 0.3 | 0.6431 |
| Weekly weight gain (g week$^{-1}$) | 1.17 ± 0.04 | 1.15 ± 0.04 | 0.6283 |
| Final biomass (kg tank$^{-1}$) | 2.71 ± 0.14 | 2.62 ± 0.07 | 0.3936 |
| Productivity (kg m$^{-3}$) | 3.04 ± 0.16 | 3.27 ± 0.09 | 0.1125 |
| Feed conversion ratio (FCR) | 1.54 ± 0.11 | 1.61 ± 0.07 | 0.3757 |
| *Performance of* S. neei | | | |
| Mean final weight (g) | 14.5 ± 5.9 | 12.8 ± 4.8 | 0.3597 |
| Final biomass (g tank$^{-1}$) | 383.3 ± 58.4 | 338.2 ± 33.0 | 0.4510 |
| Survival (%) | 93.8 ± 1.8 | 94.0 ± 2.1 | 0.6964 |
| Productivity (kg m$^{-2}$) | 1.26 ± 0.24 | 1.13 ± 0.11 | 0.4510 |

One of the concerns of growing plants in aquaponics with BFT is the management of the solids concentration in the water, as these can severely affect the roots and impact the absorption of nutrients. However, the continuous use of the settling chamber can break the microbial aggregates into small non-settleable particles, more difficult to control. In the treatment with settling, there was a thick layer of floating solids probably broken during the water pumping and sludge return to the tank. Thus, the reduction in the concentration of total suspended solids and settleable solids may be responsible for decreasing the biomass of nitrifying bacteria in this treatment (Hu et al., 2015; Pinheiro et al., 2017). In the treatment with settling, the reduction of the TSS concentration led to the gradual increase of TAN and $NO_2$ in the water, since lower solids levels may disrupt the nitrification process, leading to an accumulation of these compounds (Schveitzer et al., 2024).

This study demonstrated, under the experimental conditions presented, the viability of aquaponic cultivation of *P. vannamei* and *S. neei* in biofloc systems without the need for a settling chamber to reduce solids before the water passes through the hydroponic bench, thereby simplifying the system. Moreover, the absence of a settling chamber before the hydroponic bed resulted in more stable water quality parameters. Although the system without the settling chamber exhibited higher concentrations of suspended solids, this did not hinder the growth of either the plants or the shrimp. Nevertheless, the eventual use of a settling chamber remains necessary to maintain suspended solid levels within a range suitable for marine shrimp cultivation, particularly in aquaponic systems with longer production cycles.

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# TAILORING DIETS FOR THE EARLY STAGES OF FLATFISH: REVIEWING LATEST FINDINGS ON SOLE AND TURBOT LARVAE NUTRITION

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## Introduction

Flatfish species such as Senegalese sole (*Solea senegalensis*) and turbot (*Scophthalmus maximus*) have emerged as valuable niche species in Southern-European aquaculture, primarily due to their high-quality white flesh and elevated market value. Nonetheless, these species have some significant behavioural, anatomical and physiological variations. Namely, both present differences in feeding behaviour and digestive tract anatomy, already noticeable from the post-larval stage, with sole being more passive, feeding on the bottom of the tanks and displaying an elongated digestive tract typical of an omnivorous species. Contrarily, turbot actively feeds on water surface and exhibits a well-developed stomach and a shorter intestine, typical traits of a carnivorous species. Moreover, considerable research and development efforts over the past two decades have led to notable advancements in sole larval rearing techniques, including the refinement of weaning protocols, improvements in microdiet quality, and optimization of zootechnical procedures (Pinto et al., 2018). In contrast, the literature on nutritional strategies and feeding protocols during the early developmental stages of turbot remains relatively scarce. The present study aims at reviewing the latest efforts conducted on the road to create specific microdiets for sole and turbot larvae. To this end, this study aims at demonstrating how tailored microdiets can help tackling the nutritional challenges faced by each species during the early developmental stages.

## Materials and methods

Experimental trials were conducted during the first weeks of development of sole and turbot, ranging from 25 to 70 days after hatching (DAH). In these trials, larvae were reared in triplicate tanks under standard zootechnical conditions and fed *ad libitum* on experimental diets comprising of each trial. Trials focusing on macronutrients evaluated sources (marine vs plant-based) and levels of protein (55-61%) and lipids (12-17%). In terms of micronutrients, dietary calcium to phosphorous (Ca:P) ratio was investigated to understand its effect on skeletal malformations and body conformation of sole (e.g. slender vs round phenotype). In addition, the adequacy of feed additives (e.g. binder levels) and functional ingredients (e.g. curcumin) were also evaluated for both species. At the end of experimental trials fish were analysed for growth parameters (weight, length and relative growth rate), survival, and feed conversion ratio, to evaluate the biological efficacy of dietary treatments. The physical properties of microdiets (e.g. floatability) and leaching of water-soluble nutrients were also monitored on the different experimental trials to ensure their adequacy for the feeding behaviour of each species.

## Results

Results from the experimental trials showed different scenarios for sole and turbot, not only in terms of preferential ingredients to be included in microdiets, but also on sources and levels of macro/micronutrient to be adopted. Sole showed no preference between dietary protein sources (fish, squid, krill and plant-based diets), increasing growth performance when high levels of protein and medium levels of lipids were included in the diet. In addition, graded levels of dietary Ca:P ratios did not affect sole development in terms of growth performance, skeletal malformations or body conformation. The inclusion of high binder levels also increased sole growth performance, with these being negatively correlated to leaching of dietary water-soluble nutrients. In terms of functional ingredients, it was observed that dietary curcumin supplementation boosted sole growth performance. Diet physical properties were also shown to be extremely relevant for sole, as depressed growth and low survival was found when fish were fed a commercial diet with high floatability and residence properties at water surface.

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On the other hand, turbot did not tolerate high levels (>30 %; feed basis) of squid meal in the diet, exhibiting good growth performances when the main protein sources were fishmeal, krill meal or a mixture of marine and plant-based proteins. Contrarily to sole, turbot growth was not affected by diets with protein levels equal or above 55 %, or when dietary lipids were at medium or high levels. Opposed to sole, diet binder levels did not play a key role in turbot growth, with fish showing similar performances at low and high levels. Dietary curcumin supplementation did not play a significant role in boosting turbot growth but had a positive effect on larval total antioxidant capacity.

**Discussion**

Senegalese sole and turbot showed a different response to the nutritional composition of microdiets during the early developmental stages. As an omnivorous species, sole is apparently more eclectic in its dietary preference, showing a good response to several sources of dietary protein. However, continuous research showed by this study on the effects of different dietary nutritional compositions indicates the species tends to prefer high-protein diets and moderate lipid levels, benefiting from diets with a high binder inclusion level that retains water-soluble nutrients for a prolonged period. This feature is particularly important due to its passive feeding behaviour, with a positive effect on water quality. These clear nutritional preferences justify the considerable amount of research conducted during the past two decades to optimise the nutritional composition of sole diets (e.g. Pinto et al., 2016; Canada et al. 2017; Pinto et al., 2018). On the other hand, turbot seems to require a diet with a lower degree of specialization during the early developmental stages, as it is more tolerant to nutritional changes without a significant loss of performance. Combined with its voracity and active feeding behaviour, it is also perceptible why a lower research effort has been conducted on nutritional preferences and weaning of the species. Nonetheless, it is clear that a unique diet will not be optimised for both species, as nutritional composition and diet physical properties such as floatability have a preponderant role on the performance of sole and turbot during the early developmental stages. To this end, this work supports that tailoring the nutritional composition and physical properties of diets for marine fish larvae will contribute to produce high quality juveniles in aquaculture.

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# ASSESSING PUBLIC SUPPORT FOR AQUATIC ANIMAL WELFARE: INSIGHTS FROM A MULTI-NATIONAL SURVEY

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An estimated 133 billion fish and 630 billion crustaceans are farmed for human consumption annually. Despite their vast numbers, scientific knowledge about the welfare needs of aquatic species remains limited, and their welfare is often severely compromised in aquaculture.

Public attitudes play a crucial role in shaping welfare policies. To understand citizens' awareness, perceptions, and values regarding aquatic animals, an online survey of 12,301 respondents from 12 countries was conducted. Descriptive statistics were employed to study participants' responses, and statistical models were applied to identify the factors modulating support for fish-welfare legislation. Results indicate strong preferences for welfare protection, with many supporting better protections for farmed fish. Many respondents also indicated they would pay premium prices for such products, highlighting the potential for market-driven welfare improvements. Additionally, statistical modelling revealed that the strongest predictors of participants' support were the country of residence, knowledge of fish farming practices, and beliefs about fish sentience and cognitive capacities.

Nevertheless, challenges persist: many citizens remain unaware of aquaculture and welfare issues, and there are discrepancies in perceptions of aquatic versus land animals. Addressing these barriers is crucial for fostering greater concern for aquatic animal welfare.

# CATCHES AS A TOOL FOR ESTIMATING AQUACULTURE ESCAPES. THE CASE OF THE CANARY ISLANDS BETWEEN 2006 AND 2020

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## Introduction

Marine cage aquaculture establishes multiple interactions with its environment, which can have an impact on the local fishing sector, both ecologically and socioeconomically, and can affect the dynamics and sustainability of fishing activity. One of the main impacts on the marine environment is the escape of organisms (Toledo Guedes et al., 2024).

The Canary Islands are a volcanic archipelago located in the Atlantic Ocean, northwest of Africa, consisting of eight main islands and with a subtropical climate. Aquaculture on these islands has been taking place mainly since the 1990s, with the farming of non-native species in the area such as sea bream (*Sparus aurata*) and sea bass (*Dicentrachus labrax*).
The best method to combat escapes is for the professional fleet to capture these escaped individuals. As time passes since an escape, there is greater dispersion of these organisms (Atalah et al., 2024). The capture of escaped organisms usually occurs in shallow areas near aquaculture facilities (Toledo-Guedes et al., 2014).

## Objectives

This study has two main objectives. The first is to assess whether the increase in sea bream and sea bass production in the Canary Islands is reflected in the number of catches reported by commercial vessels. The second objective is to analyze whether the geographical distribution of aquaculture activity affects the quantity of these species caught in the region.

## Materials and methods

Data on aquaculture production and catches of sea bream (*Sparus aurata*) and sea bass (*Dicentrachus labrax*) in the Canary Islands were used. The catch data came from the General Secretariat for Fisheries, while the production data were obtained from APROMAR's annual reports.

Regarding the data processing for the first objective, the annual production and extractive fishing data for the islands as a whole were analyzed jointly for the period 2006–2020. Initially, a simple linear regression model was fitted. Since the relationship showed a poor fit and was not statistically significant, a logarithmic transformation was applied to both variables to improve the linearity and homoscedasticity of the residuals. The performance of the models was evaluated using the Akaike (AIC) and Bayesian (BIC) information criteria, selecting the former as optimal.

Regarding the second objective, sea bream and sea bass catches broken down by island and year (2006–2020) were used. The data were grouped by island and year, and Bray–Curtis distances were then calculated to estimate the dissimilarity in catch composition between islands. A PERMANOVA analysis was then applied to evaluate significant differences between years and islands. Post hoc comparisons were then made between pairs of islands using the Bonferroni correction to control for type I error.

## Results and discussion

Regarding the first analysis, the logarithmic linear regression model (R2= 0.543) explains 54.3% of the variability. There is a strong and positive relationship between production and catch: higher production leads to higher catch, in a significant and non-linear way. Despite this, with the increase in production, the explained variability is not excessively high, as other very decisive factors such as storms or sabotage may influence it. Storms can have a major effect on variability, as cages are more likely to break and allow organisms to escape after such extreme events (Toledo-Guedes et al., 2014 a,b) or acts of sabotage (Atalah et al., 2024).

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As for the second analysis, the results indicated that there were no significant differences in the composition of the catch between the different years analyzed (2006–2020) ($F = 0.76$, $p = 1.00$). However, significant differences were found between the islands ($F = 4.78$, $p = 0.001$), suggesting that the total catch of escaped individuals varies significantly between the different islands. Despite this, this model explains 14.7% of the variation, with the remaining 85.3% of the variability attributed to residual variability not explained by the factors included. These results indicate that geographical location, i.e., the islands, is a determining factor in the difference in the catch of escaped organisms. The results of the post-hoc tests with Bonferroni correction revealed significant differences in the composition of catches between most of the pairs of islands analyzed. The most notable differences were observed between Gran Canaria and La Palma ($F = 7.27$, adjusted $p = 0.015$), as well as between La Palma and Lanzarote ($F = 7.00$, adjusted $p = 0.015$). In addition, Fuerteventura showed significant differences with all islands. However, no significant differences were found between Gran Canaria and La Gomera after correction (adjusted $p = 0.09$). These results indicate significant spatial variability in catch composition across the archipelago.

These differences may be due to the distribution of aquaculture facilities throughout the archipelago. During the years analyzed, La Palma, Tenerife, Gran Canaria, and Lanzarote have farmed sea bream and sea bass in marine cages. Fuerteventura and La Gomera are the only two islands that have catches and have not farmed these species on the island, mainly due to the presence of these organisms from marine cages. Fuerteventura shows significant differences from all the other islands, which may be due to the absence of aquaculture and its greater distance from all the other western islands. These results reinforce the existence of clear spatial differences in the composition and quantity of catches between most of the islands in the archipelago.

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# VERIFISH: THE SUSTAINABILITY INDICATOR FRAMEWORK TO COMMUNICATE RESPONSIBLE AQUAFOOD PRODUCTION AND CONSUMPTION PATTERNS

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## Introduction

Today's seafood sector operates within a highly intricate ecosystem where sustainability encompasses a broad spectrum of interconnected dimensions, including environmental impact, social equity, economic viability, and nutritional value. Understanding and effectively communicating these multifaceted aspects are crucial for encouraging responsible practices, safeguarding marine resources, and ensuring equitable benefits for all stakeholders. In this context, the Horizon Europe VeriFish project[1] is providing an innovative system based on existing EU and global sources to meet the growing need for transparent, standardised, and actionable metrics for communication in the aquafood sector.

## The VeriFish Indicator Framework

The VeriFish Indicator Framework[2] addresses key challenges in data accessibility, communication and informed decision-making by supporting provision of trustworthy and verifiable information on the sustainability of aquafood products. It is structured around three pillars: (a) environmental, (b) nutrition and health and (c) social and economic. The framework is supported by a rich collection of trustworthy data sources, each contributing verifiable information aligned with the indicators defined under the three pillars.

## Key Indicators for Fisheries and Aquaculture

The environmental pillar focuses on minimising ecological impacts and promoting sustainable use of resources in both capture fisheries and aquaculture. It encompasses a broad range of indicators that reflect climate and ecological impacts, such as bycatch, gear loss, and carbon footprint, as well as stock status, animal welfare, and resource use efficiency. These indicators are informed by a variety of established and credible data sources, including the FAO Global Record of Stocks and Fisheries (GRSF), FishBase and SeaLifeBase, the IUCN Red List of Threatened Species, EMODNET Biology datasets, and WISE-Marine, among others.

**Assessing Nutritional Value in Sustainable Seafood**

The nutrition and health pillar underpins the role of aquafood in supporting human health and meeting dietary needs. Indicators are based on nutrients selected for their relevance to public health including protein content, omega-3 fatty acid, sodium, and micronutrients. These indicators not only reflect the nutritional significance of aquafood, but are also broadly applicable across a wide range of species. The primary data source supporting these indicators are drawn from EuroFIR's FoodEXplorer, which provides harmonized food composition data. Additional data sources- including the FAO/INFOODS Global Food Composition Database for Fish and Shellfish (uFish), various data collections from the World Health Organisation (WHO), and others- complement indicators for this pillar.

**Labour, Governance, and Equity in Aquafood**

The social and economic pillar underscores the importance of fair, inclusive and responsible practices across all stages of fisheries and aquafood production. Its indicators are organized into thematic sub-pillars: governance, labour practices and rights, health and safety of workers and issues related to discrimination and indigenous peoples. These indicators are primarily grounded in the conventions of the International Labour Organization (ILO), and their ratification status by individual countries. In addition, complementary data sources- such as the Global Slavery Index, Transparency International Corruption Perceptions Index, Flags of Convenience lists, and others- are leveraged to enhance the depth and contextual relevance of the indicators.

**VeriFish prototype mobile application**

The VeriFish framework provides measurable indicators for capture fisheries, aquaculture, and nutrition, enabling assessment of key sustainability metrics such as biodiversity conservation, carbon emissions, resource efficiency, and public health. These indicators form the basis of a prototype web application[3] that serves as a user-centric gateway to the VeriFish framework of indicators. It facilitates communication of sustainability by actors and enables consumers to make more informed decisions based on their priorities.

**Conclusions**

By standardising sustainability reporting and enhancing value-chain communication, the VeriFish framework can foster consumer trust and empowerment, promote responsible 'advertising' practices, and pave the way for diverse, sustainable consumption.

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# EXPLORING ROBUSTNESS BIOMARKERS THROUGH FIN OMICS IN THREE EUROPEAN SEA BASS POPULATIONS

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## Introduction

Environmentally induced changes in DNA methylation have been proposed as potential contributors to short-term adaptive responses to changing environments. To investigate whether epigenetic changes are involved in the resistance to temperature as an environmental stressor, we analyzed three genetically distinct European sea bass populations—Atlantic (AT), Western Mediterranean (WM), and Eastern Mediterranean (EM)—produced by artificial fertilization and reared under common garden conditions in a relatively warm thermal regime representative of the Eastern Mediterranean (rEM). The primary objective of this study was to characterize the fin transcriptome and methylome to determine whether population-level differentiation is evident in these two omic layers. We then aimed to assess associations between omic patterns and phenotypic traits related to robustness, defined here as the capacity to resist environmental stressors and mitigate negative impacts on physiological performance and overall well-being.

## Materials and Methods

After one year of exposure to the rEM thermal regime, 612 fish were slaughtered, and phenotypic data were collected, including body weight, body length, liver and gonad weight. Photographs of whole animals were also taken to allow later scoring of fin erosion level. From these, 72 individuals were randomly selected for transcriptome and methylome analyses. RNA and DNA were co-extracted from the same fin tissue using the Quick DNA/RNA Mini Prep Plus Kit. For transcriptomic analysis, RNA from these individuals was equimolarly pooled into 18 libraries (six per population), stratified by sex and population. For DNA methylation analysis, 12 equimolar pools (four per population) were prepared using only high-quality DNA. RNA-seq libraries were sequenced at ~30× coverage, while whole-genome DNA methylation was assessed using Enzymatic Methyl-seq (EM-seq) at ~25× coverage. Following quality control and bioinformatic processing, principal component analysis (PCA) was performed, differentially expressed genes (DEGs) were identified using DESeq2, and differentially methylated regions (DMRs) were detected using the MethylKit package in R.

General linear models are being used to assess whether expression and methylation biomarkers can be associated with phenotypic traits of interest linked to robustness. Additionally, we are working to determine which of these DMRs are influenced by underlying single nucleotide polymorphisms (SNPs) and which may constitute environmentally-driven epigenetic markers.

## Results and Discussion

We observed significant differences among populations for several phenotypic traits, including condition factor and hepatosomatic index, which are associated with fish robustness. Transcriptome analysis identified 140 DEGs between EM and AT, 135 between WM and AT, and 119 between EM and WM (adjusted $P < 0.05$; $|\log_2 \text{fold change}| \geq 0.5$). Genes upregulated in the Mediterranean populations (EM and WM) were mainly associated with basal stress responses, whereas those upregulated in the Atlantic population (AT) were related to rapid stress response mechanisms, indicating population-specific molecular strategies to cope with warm environments. PCA of the methylome revealed significant differences between populations along the first three principal components. Differential methylation analysis identified 14,993 DMRs (EM vs AT), 8,391 (WM vs AT), and 4,983 (EM vs WM).

*(Continued on next page)*

Further downstream analyses—linking DMRs and DEGs and phenotypic traits—will help pinpoint candidate biomarkers of robustness under the relatively warm regime representative of the species' environmental range.

**Conclusions**

This study identifies candidate biomarkers of robustness from non-lethal fin tissue, contributing to the assessment of population vulnerability and promoting more sustainable aquaculture practices. It also enhances our understanding of genotype–environment interactions, local adaptation, and the potential impact of climate change on European sea bass populations.

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# BEYOND THE DOCK: CULTIVATING EARLY STAGES OF THE ASCIDIAN *Styela plicata* FOR A SUSTAINABLE MARINE ECONOMY

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## Introduction

The necessity for novel sources of natural products is increasing, positioning marine organisms as promising sources of sustainable food and biomaterials. Ascidians (Phylum: Chordata, Subphylum: Tunicata), or 'sea squirts', are marine filter-feeding invertebrates known for their rich chemical diversity and potential biotechnological applications (Palanisamy et al., 2017). Notably, they are the only known animals capable of producing cellulose (Xiaoyu et al., 2023). *Styela plicata* is a solitary, cosmopolitan ascidian often found in high densities on artificial structures in harbors and marinas. It is also recognized as a species that can interfere with aquaculture operations by fouling equipment and competing with farmed or native filter feeders (Pineda et al., 2013). It thrives in a wide range of environmental conditions, including high temperatures, salinity, and pollution (Platin & Shenkar, 2023), making it a strong candidate for aquaculture. In South Korea, *S. plicata* is consumed as a traditional seafood, valued for its high protein content and bioactive compounds with antioxidant and antimicrobial properties (Lambert et al., 2016). However, cultivation practices remain limited, and no standardized inland protocol has been established for this species to date. Moreover, individuals are often collected from highly polluted areas, which may affect their nutritional value. During a previous study (Platin, 2022), *S. plicata* demonstrated rapid growth and high reproductive output in a closed-water system, supporting its feasibility for scalable aquaculture. Notably, *S. plicata* produces a thick outer tunic (an external layer that protects the animal's soft body) composed of approximately 60% cellulose. Their cellulose is highly crystalline and exhibits exceptional mechanical, electrical, and magneto-responsive properties, positioning it as a promising source of tunicate nanocellulose (t-CNC) for industrial applications (Xiaoyu et al., 2023). This study introduces a controlled cultivation protocol for the early life stages of *S. plicata*, providing a basis for evaluating its potential in future applications related to food production and biomaterials. Given its physiological resilience and ability to grow in nutrient-poor conditions, *S. plicata* emerges as a promising candidate for resource-efficient marine cultivation targeting food and industrial biomaterials.

## Materials and methods

This study consisted of a series of controlled experiments designed to evaluate the growth performance and survival of *Styela plicata* juveniles under various rearing conditions, including food type (two algae and their mix), light intensity (low light *vs.* complete darkness), feeding frequency, and five different temperatures. All experiments were conducted in both Closed Water Systems (CWS) and Running Seawater Systems (RSS), except for the temperature experiment, which was performed exclusively in RSS. At the beginning of each trial, 5 to 13 adult individuals were collected from the field. Artificial fertilization was performed to produce same-age juveniles from the same parents, ensuring synchronized feeding across replicates. Survival and growth rate were measured weekly and served as the primary performance parameters, with 180 to 360 juveniles per experiment, depending on the trial. Each experiment lasted one month, except for the food type and concentration trial, which was conducted over a period of three and a half months.

## Results and discussion

Juveniles of *Styela plicata* showed high survival in RSS when fed *Nannochloropsis* at the lowest concentration, with 75 ± 8.3% survival and an average size of 2.9 ± 0.2 mm after three and a half months. A mixed diet of *Isochrysis galbana* and *Nannochloropsis* at the highest concentration resulted in 83.3 ± 14.4% survival and a larger size of 3.3 ± 0.6 mm. For subsequent experiments, *Nannochloropsis* at the lowest concentration was selected as the standard feed due to its favorable survival rates and cost-efficiency. After one month, survival with *Nannochloropsis* X reached 100% in CWS and 77.7 ± 9.6% in RSS. Temperature had a clear effect on growth. The highest average size was recorded at 26 °C (840 ± 94 µm), with significantly larger individuals ($p < 0.05$) compared to 16 °C (581 ± 40 µm), 20 °C (501 ± 175 µm), and 30 °C (585 ± 65 µm). Survival was

*(Continued on next page)*

highest at 16 °C (72.2 ± 33.6%) and declined sharply at both 20 °C (38.9 ± 17.3%) and 24 °C (38.9 ± 5.3%). These results highlight a trade-off between growth and survival, emphasizing the need to adjust rearing conditions to production goals. *S. plicata* demonstrates strong potential for controlled aquaculture, with feeding regime and temperature identified as key factors. The use of *Nannochloropsis* as a single-species feed supports both biological efficacy and economic feasibility for scalable cultivation.

**Conclusion**

The protocol developed in this study is optimized for the early stages of *S. plicata* and provides a solid foundation for its future use in aquaculture as a source of dietary supplements and cellulose for industrial applications. Although additional research is needed to refine long-term rearing strategies and evaluate later life stages, our results highlight the species' high reproductive capacity and its compatibility with open seawater systems. Notably, *S. plicata* was successfully cultivated in nutrient-poor Eastern Mediterranean seawater, demonstrating its robustness and potential for low-input marine production. Future studies should explore the species' value as a dietary supplement and as a sustainable source of tunicate cellulose following controlled aquaculture.

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# MACRO- AND MICROELEMENT CONTENT IN OYSTERS (*Ostrea Edulis* L.) CULTIVATED IN THE MALI STON BAY, ADRIATIC SEA

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## Introduction

Oysters are among the most commonly farmed marine species and are native to almost all ocean regions with the exception of the polar regions. They are prized as a delicacy and play an important role in global seafood consumption. In recent years, growing awareness of their nutritional value has led to an increase in demand, with oyster production increasing by an average of 7.8% per year over the last three decades. Compared to finfish, shellfish generally contain a higher protein content, and oysters are no exception. They are also rich in macroelements (Na, K, Ca, P and Mg) and trace elements (Cr, Co, Cu, F, Br, I, Fe, Se, Zn and Mn), some of which, such as iron, copper and zinc, play a crucial role in various biological systems. The aim of this study was to investigate the seasonal variations in the mineral composition of shallow oysters (*Ostrea edulis* L.) from the Bay of Mali Ston in the Adriatic Sea.

## Materials and methods

Samples were taken each month over a seven-month period and analyzed by flame atomic absorption spectroscopy (200 Series A4 with SPS 4 Auto-Sampler, Agilent Technologies, USA) to determine the content of selected macro- and micro- elements.

## Results

The results show that the macroelements (Figure 1) exhibit greater seasonal fluctuations, while the microelements (Figure 2) remain relatively constant. The average content of minerals in all samples was as follows: 6011 mg/kg for Na, 2613 mg/kg for Ca, 3533 mg/kg for K, 858 mg/kg for Mg, 275 mg/kg for Zn, 44 mg/kg for Fe, 17 mg/kg for Cu and 1586 mg/kg for P. Although specific fluctuations were observed in individual months, the overall constancy of the microelements indicates consistency in mineral content despite environmental changes.

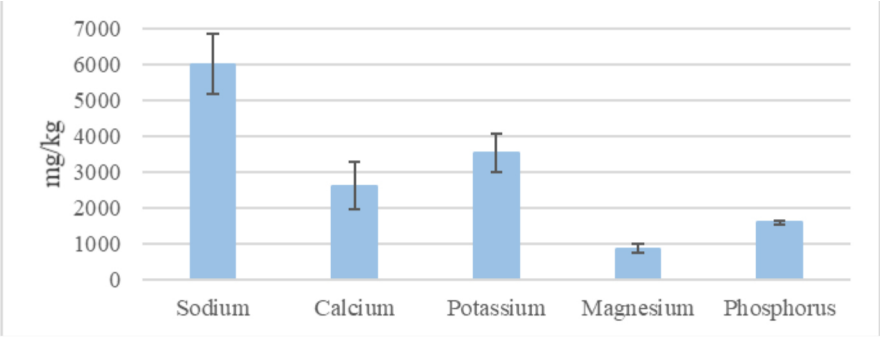


Figure 1. Macroelements in oysters – mean concentrations with standard deviations

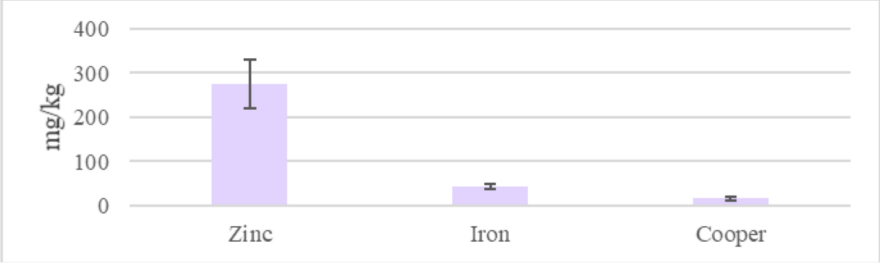


Figure 2. Microelements in oysters - mean concentrations with standard deviations

# LOW OR HIGH, WHERE DO REDCLAWS LIE? SHELTER USE IN CRAYFISH CULTURE

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## Introduction

The redclaw crayfish (*Cherax quadricarinatus*) is a freshwater crayfish native to northern Australia and southern Papua New Guinea. It has gained increasing attention in global aquaculture due to its fast growth, high market value, and adaptability to a wide range of environmental conditions.

In confined environments, shelter plays a key role in reducing aggression, improving survival, and enhancing overall welfare. Redclaw crayfish are naturally territorial and exhibit increased aggressive interactions when shelter is limited, especially after molting. This can lead to injuries, stress, reduced growth performance, and decreased survival.

Can redclaw crayfish be cultured in higher tanks with less surface area, containing vertical shelter places? This study investigates the use of vertical shelter, when provided, and the effects on the behavior, growth, and survival of redclaw crayfish reared in indoor systems, with the aim of optimizing culture conditions for sustainable and productive aquaculture operations with attention to animal welfare.

## Materials and methods

Crayfish were kept at 27°C in six 800 L flat-bottomed square tanks. A photoperiod of 16h light/8h dark was applied. All tanks were connected to a RAS system with mechanical and biofiltration. 15 and 30 crayfish were stocked in tanks with low (five layers) and high (ten layers) tube bioblock shelter types respectively, in order to provide each animal with the same number of shelter tubes. Crayfish were fed once daily with a feeding level of 4% of total biomass. The trial ran 12 weeks and both treatments were performed in triplicate.

Every three weeks, all live animals were counted and weighted. Average Body Weight (ABW) and survival percentage were calculated.

During the first nine weeks, shelter occupation was recorded on three days per week. During each of these 27 observation moments, shelter occupation in one low shelter and one high shelter tank was recorded by counting the number of crayfish in each tube.

## Results

Redclaw crayfish prefer to occupy the lower tube layers, with a clear preference for the bottom layer. During the 27 observation moments in total, 75 and 36 crayfish were observed in the lowest tube layer in high and low shelters respectively. Note that the stocking numbers of crayfish in the high shelter tanks were twice the numbers in the low shelter tanks. The three top layers in the high shelter tanks had a low occupation rate. The heat map below (right) shows how many crayfish were observed per layer during the 27 observations in total in high and low shelter tanks.

The graph below (left) shows that the Average Body Weight (ABW) evolved from 7.91g ± 0.18g and 8.09g ± 0.12g in low and high shelter tanks respectively on week 0 to 23.52g ± 0.37g and 20.70g ± 1.97g in low and high shelter respectively on week 12. From the sampling on week 6 onwards, the ABW was significantly higher in low shelter tanks compared to high shelter tanks ($p$-value $< 0.05$).

Surprisingly, no significant effect of low and high shelter on survival rate could be shown

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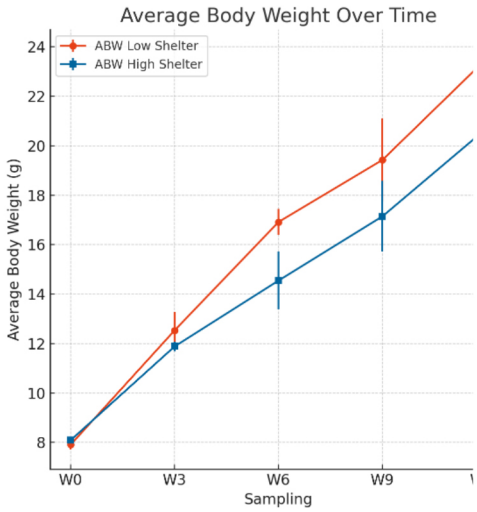


Fig. 1 Average body weight of crayfish in high and low shelter tanks. Error bars indicate standard deviation between tank replicates.

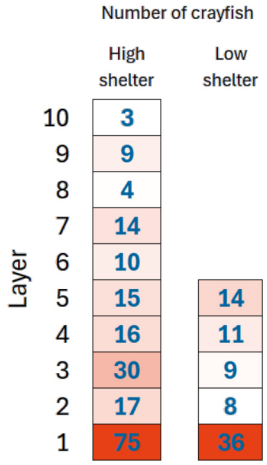


Fig. 2 Heat map showing total number of crayfish observed per layer of tubes in high and low shelter tanks during all observations.

**Discussion**

To provide an equal number of shelter tubes per crayfish, high shelter tanks contained twice the crayfish density per square meter bottom surface compared to low shelter tanks.

However, due to the low occupancy of upper tubes in high shelter tanks, competition was concentrated at the lower levels, likely intensifying interactions over limited bottom surface area and shelter tubes close to the bottom.

Increased competition in high shelter tanks may have led to more frequent and complex dominance interactions, potentially suppressing growth in subordinate individuals. In contrast, crayfish in low shelter tanks likely experienced more consistent access to feed and more efficient shelter use, facilitating simpler spatial dynamics and improved growth. However, no significant difference in mortality, primarily attributed to aggressive interactions and cannibalism, was observed between treatments.

These findings indicate that reducing bottom surface area by increasing vertical shelter complexity can negatively affect growth performance in indoor redclaw crayfish culture. Further research is recommended to evaluate alternative shelter designs and stocking strategies that optimize bottom space utilization and support growth.

# SUPLEMENTATION OF THE MICROALGAE *Arthrospira platensis* IN THE NURSERY PHASE OF PACIFIC WHITE SHRIMP (*Penaeus vannamei*, BOONE 1931) IN A BIOFLOC SYSTEM

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This study evaluated the effect of adding dry biomass of the microalgae *Arthrospira platensis* (*Spirulina*) in to the water on shrimp growth, water quality and proximal composition of *Penaeus vannamei* post-larvae in a biofloc system. Four treatments were tested: (BFT) control without *A. platensis*; (*Sp40*) biofloc with 40 mg $L^{-1}$ mg/L of *A. platensis*; (*Sp50*) biofloc with 50 mg $L^{-1}$ of *A. platensis*; and (*Sp60*) biofloc with mg $L^{-1}$ of *A. platensis*. The shrimp with an initial average weight of 0.015 ± 0.009 were stocked at a density of 2000 post-larvae $m^3$ in polyethylene tanks with a useful volume of 50L and fed commercial feed. The experiment lasted 35 days and assessed zootechnical parameters, water quality and the proximal composition of the shrimp and the biofloc. Treatments with *A. platensis* showed improved biomass gain, better survival, and feed conversation rate, with a higher protein content in the shrimps when compared to control. The water quality parameters were significantly influenced by *A. platensis*, with reductions in pH and alkalinity in the treatments with the additions of *Spirulina*, particularly in *Sp60*, which showed the lowest values. Nitrate and phosphate concentrations increased in all *Spirulina* treatments compared to the control, with the highest values observed in the *Sp50* and *Sp60* treatments. However, all water quality parameters remained within tolerance levels for shrimp growth. Thus, among the treatments tested, the treatment with addition of 50 mg $L^{-1}$ of *A. platensis* powder positively influenced the survival and growth of the shrimp and improved their nutritional composition in the nursery phase of *P. vannamei* in the biofloc system.

# INFLUENCE OF MALE GENETIC BACKGROUND ON TEMPERATURE-DRIVEN DEVELOPMENTAL OUTCOMES IN ATLANTIC SALMON *salmo salar*

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## Introduction

Climate change is a major anthropogenic driver of biodiversity loss, intensifying extreme weather events such as floods and heatwaves (Heino et al., 2009). In freshwater systems, rising temperatures threaten cold-water species like Atlantic salmon (*Salmo salar*) (Buisson et al., 2008; Comte & Grenouillet, 2013). As ectothermic stenotherms, salmon are highly sensitive to temperature, especially during embryogenesis, which is optimal between 7–8 °C (Angilletta et al., 2002; Fraser et al., 2015). Hence, even slight thermal deviations can disrupt biochemical and metabolic processes, affecting growth, reproduction, and behaviour (Jonsson & Jonsson, 2009). Elevated incubation temperatures (>8 °C) have been linked to reduced survival, altered development, and increased abnormalities in salmon larvae (Kamler, 2002).

In Atlantic salmon populations, two male reproductive strategies are observed: migratory (anadromous) males, and precociously maturing males that reproduce in freshwater during their first (age 0+) or second (age 1+) year without seaward migration. This alternative tactic enhances resilience in harsh environments and is thought to result from interactions between genetic predisposition and environmental factors such as temperature-driven growth (Vladic et al., 2010; Martinez et al., 2000). With projected river warming, increased frequencies of precocious maturation may shift population structure toward freshwater residency, potentially altering genetic dynamics. These dynamics are particularly relevant for conservation aquaculture and the design of effective restocking strategies that aim to preserve genetic diversity and ensure the long-term viability of restored populations.

This study aims to evaluate how male genetic background modulates the effects of elevated water temperatures during embryonic development on key traits critical to Atlantic salmon restoration. Specifically, it will assess the impact of increased incubation temperatures on: (i) juvenile stress-related behaviour and (ii) immune system functionality.

## Material and Methods

Six wild anadromous male Atlantic salmon (*Salmo salar*, 5–6 years old) were captured during their 2024 upstream migration using a trapping system at the Lith fish ladder on the Meuse River (Netherlands). They were held in all-male tanks at the CoSMOS (Conservatoire du saumon mosan) hatchery in Érezée, Belgium (50°18'21.78"N, 5°32'33.61"E) until the breeding season. Additionally, six hatchery-reared precocious parr males (age 1+) raised at CoSMOS were used for reproduction.

Eggs were collected from farmed females (age 4+), pooled, and divided into batches of 300 eggs. Each batch was fertilized using fresh sperm from one of the 12 males to obtain a standard sperm-to-egg ratio of $10^6$. Fertilized eggs were incubated at the University of Namur in a dark, temperature-controlled room at 11 °C. After yolk sac resorption, larvae were transferred to larger tanks for free swimming and feeding to apparent satiation. Following two weeks of feeding, the juveniles underwent a standardized startle response test. Subsequently, half of the individuals were exposed to a bath infection with *Aeromonas salmonicida* achromogenes at a concentration of $1.41 \times 10^9$ CFU/mL for 12 hours at 11°C, while the other half served as an uninfected control group. Fish were then euthanized for liver and brain tissue collection.

RNA-seq was performed to identify differentially expressed genes (DEGs) between paternal origins. Selected genes related to immunity and stress were further analysed using RT-qPCR. In addition, RT-qPCR on telomere will be performed to assess the impact of genetic on chronic stress.

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**Results and discussion**

Preliminary results indicate generally low survival rates across all groups, with an average of 37.6 (min: 25.9, max: 57.5) for from anadromous males and 33.5 (min: 25.7, max: 42.8) f or offspring from precociously maturing parr. In the behavioural trials, we anticipate that juveniles fathered by anadromous males will demonstrate faster escape responses under stress. Finally, transcriptomic analyses are still underway, however, we expect to identify distinct gene expression patterns between offspring from the two male types, particularly in pathways related to stress response and immune function. These results would suggest that paternal reproductive strategy influences offspring resilience to early-life thermal stress, with implications for Atlantic salmon adaptation under future climate change scenarios.

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# REGULATORY READINESS IN AQUAFEED INNOVATION: BRIDGING NUTRITION, POLICY AND PRACTICE

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**Introduction:**
Aquafeed innovation is increasingly shaped by sustainability goals and the search for alternatives to traditional protein sources such as fishmeal. New ingredients — including agro-industrial by-products, insect meals, microalgae, single cell proteins (SCPs), and fermentation-derived biomass — offer promising nutritional and environmental advantages. However, their integration into commercial aquafeeds is often constrained by regulatory bottlenecks and unclear legal classifications under the European Union feed legislation.

One key barrier lies in the classification of feed ingredients within the EU regulatory framework. While placing a new feed material on the market may not require EFSA evaluation, this changes when the product is either considered a feed additive — subject to mandatory EFSA authorisation — or may be interpreted as a "novel" feed material under current regulatory practice, due to lack of historical use or innovative processing. Although no formal EU definition of "Novel Feed" exists, such cases will trigger an EFSA authorisation process. In both cases, authorisation processes can be complex and time-consuming, delaying innovation in feed formulation.

This presentation draws on internal formulation work conducted at SPAROS during the CIPROMED project, sharing lessons learned from the screening and evaluation of novel ingredients under real-world regulatory and nutritional constraints. The approach aligns with CIPROMED's mission to valorise local biomass and develop circular, sustainable protein sources within Mediterranean food systems.

**Internal approach**
To contextualize regulatory constraints, it is important to distinguish two common bottlenecks: (1) ingredients not listed in the EU Catalogue of Feed Materials (Reg. (EU) 68/2013) for aquaculture species — which may be permitted for terrestrial animals but not for fish — and (2) ingredients without documented history of use in aquafeed, or which involve new production processes, thus triggering additional legal scrutiny.

A structured, practice-oriented screening process was implemented to support internal ingredient triage and early-stage formulation planning, aiming to reduce regulatory and technical risk. Although scientific assessment typically begins with nutritional suitability, regulatory feasibility was screened early in this case to avoid investment in ingredients unlikely to reach market approval. The process included five sequential filters:

(1) Regulatory status (Catalogue listing, Registration process (if new ingredient), species and substrate restrictions);
(2) Nutritional suitability (composition, literature-based digestibility, anti-nutritional factors);
(3) Feed safety (compliance with legal undesirable substances thresholds);
(4) Technical feasibility (process compatibility, ingredient stability);
(5) Strategic relevance (alignment with sustainability and product innovation goals).

Ingredient performance modelling was supported by FEEDNETICS™, a nutritional simulation tool that estimates growth and nutrient retention using user-defined inputs such as feed intake, digestibility coefficients, proximate composition, fatty acid, and amino acid composition. This supported decision-making when in vivo data were limited or unavailable.

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**Results and Discussion**

This framework enabled risk-based screening in early formulation stages:

- Oriental fruit fly larvae (*Bactrocera dorsalis*) reared on mixed vegetal waste:

  This ingredient shows promising nutritional value but is currently not authorised under Regulation (EU) 2017/893, which limits insect feed use to specific species and approved substrates. Both the insect species and the vegetal substrate fall outside current regulatory allowances. However, this case underscores the importance of aligning insect rearing practices with existing approvals or supporting novel applications through EFSA dossiers.
  (Filters 1 and 5 – Regulatory status, Strategic relevance)

- Mediterranean fruit fly (*Ceratitis capitata*) larvae reared on olive pomace:

  Olive pomace is a sustainable agro-industrial by-product, widely studied and legally acceptable as a substrate. However, this insect species is not among the seven approved for feed use in aquaculture. Historical data on its safe use is lacking, which would necessitate a full EFSA safety assessment under Regulation (EC) 767/2009 before commercial inclusion. This highlights an opportunity for further research and regulatory engagement. (Filters 1, 2, and 3 – Regulatory status, Nutritional suitability, Feed safety)

- Schizochytrium sp., dried biomass:

  Both dried and fermented biomass forms are listed as feed materials in the EU Catalogue (e.g. 13.1.5) and can be used in aquafeeds under general feed law. Oil extracts — such as those commercialised in the aquafeed market — are treated as feed materials, not feed additives, and thus do not require pre-market EFSA authorisation. Their inclusion depends on meeting safety criteria (e.g. contaminant limits) and being properly labeled. These products represent successful examples of regulatory-compatible innovation in DHA supplementation. (Filter 1 – Regulatory status; Filter 2 – Nutritional suitability)

- Spirulina (*Arthrospira platensis*):

  A well-established microalga in aquafeeds, Spirulina is listed in the EU Feed Catalogue and supported by extensive research confirming its nutritional and antioxidant properties. As aquafeed innovation evolves, claims beyond nutrition (e.g., immune modulation) or use of novel strains or extraction techniques may require further validation. (Filters 1, 2, and 4 – Regulatory status, Nutritional suitability, Technical feasibility)

- Yeast-based SCP (*Cyberlindnera jadinii*):

  A non-GMO protein-rich biomass already present in the EU Feed Catalogue (sections 13.8.1 and 13.8.3), and widely used in commercial aquafeeds. Its established safety, nutritional value, and processing consistency position it as a low-risk ingredient for inclusion in formulation pipelines. (Filters 1, 2, and 4 – Regulatory status, Nutritional suitability, Technical feasibility)

This approach helped avoid investment in ingredients with low regulatory feasibility and supported early alignment between legal, nutritional, and technical considerations.

**Conclusion**

A paradox emerges: ingredients with the highest sustainability and circularity potential — such as insect meals on alternative substrates or agro-industrial residues — are often the most constrained by regulatory frameworks. By integrating legal feasibility into early-stage formulation, this framework improves internal decision-making and enhances support for clients developing experimental feeds. These insights directly support CIPROMED's mission to foster responsible innovation and valorise underused biomass in Mediterranean aquaculture systems.

# TOWARDS SUSTAINABLE SEA URCHIN AQUACULTURE: IMTA APPLICATION WITH POLYCHAETES AND MACROALGAE

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## Introduction

Integrated Multi-Trophic Aquaculture (IMTA) promotes sustainability by combining species from different trophic levels to optimise nutrient utilisation. The sea urchin *Paracentrotus lividus*, highly valued in Southern Europe, presents slow growth rates that continue to limit its aquaculture potential. The inclusion of extractive species such as the polychaete *Hediste diversicolor* and the macroalga *Ulva rigida* may enhance system efficiency through the recycling of organic and dissolved nutrients. This study evaluated the survival and growth of *H. diversicolor* and *U. rigida* in a three-level IMTA with *P. lividus* as the fed species.

## Materials and methods

To evaluate the growth of extractive species under different IMTA configurations with *P. lividus*, a six-week trial was conducted with three treatments in triplicate, designed to compare the performance of *U. rigida* and *H. diversicolor* under separate and combined co-culture conditions: (1) *U. rigida* cultivated in static kreisel tanks (3 L available volume; 1 g $L^{-1}$ initial density) supplemented with culture medium; (2) co-culture of adult *P. lividus* and juvenile *H. diversicolor* in a recirculating aquaculture system (RAS); and (3) a full three-level IMTA integrating all species. *U. rigida* density was reset to 1 g $L^{-1}$ at the beginning of week 4. *H. diversicolor* (50 individuals per tank) was reared in a sediment layer at the bottom of 32 L tanks, with *P. lividus* (15 individuals per tank) held above in mesh containers. Sea urchins were fed a formulated dry diet. Mean environmental parameters across treatments were 18.2 °C, pH 8.5, and salinity 33.9. All organisms were weighed at the beginning and end of the trial. Growth was evaluated as specific growth rate (SGR).

## Results

Survival was 86% in polychaetes across both RAS treatments. Polychaete initial weights were 25.27 ± 3.97 mg (2) and 21.40 ± 2.62 mg (3), increasing to 0.37 ± 0.05 g and 0.39 ± 0.09 g, respectively, by the end of the trial. Polychaete SGR was 7.09 ± 0.45% $day^{-1}$ in the co-culture (2) and 7.81 ± 0.77% $day^{-1}$ in the full IMTA (3), with no significant differences between treatments. *U. rigida* showed an overall SGR of 4.06 ± 1.68% $day^{-1}$ in the control (1) and 2.01 ± 0.47% $day^{-1}$ in the IMTA system (3), although growth differences were not statistically significant. The peak weekly SGR occurred in week 2 for the static culture (5.95 ± 1.04% $day^{-1}$) and in week 6 for the IMTA treatment (3.20 ± 2.09% $day^{-1}$).

## Discussion

This study confirms the feasibility of integrating *H. diversicolor* and *U. rigida* with *P. lividus* in a three-level IMTA system. Polychaetes exhibited high survival and SGR values exceeding 7% $day^{-1}$, among the highest reported and comparable to those obtained using waste from carnivorous fish or high-protein diets. These findings highlight the nutritional suitability of sea urchin-derived organic waste for detritivorous species. Slightly higher polychaete growth under IMTA conditions may reflect indirect benefits from improved water quality, as *U. rigida* is known for its capacity to assimilate dissolved nitrogen compounds. Conversely, macroalgal growth was lower in the IMTA system compared to the static control, potentially due to limited nutrient availability and suboptimal flow conditions. *Ulva* productivity in IMTA systems depends not only on total nutrient concentrations, but also on the ratio and form of available nitrogen and phosphorus, as well as the presence of trace elements, which may limit uptake even when macronutrients are not limiting. Despite these limitations, biological performance across all components remained stable, supporting the use of low-trophic IMTA for integrated nutrient recovery and sustainable biomass production.

# INTEGRATED AQUACULTURE OF *Paracentrotus lividus* AND *Chondracanthus acicularis*: DOES URCHIN DENSITY MATTER?

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## Introduction

Integrated Multi-Trophic Aquaculture (IMTA) is recognized as a sustainable method in aquaculture, bringing together species from various trophic levels to enhance nutrient utilization, minimize environmental impact, and transform waste materials into valuable, edible products. Therefore, IMTA has the potential to promote sustainable farming by improving efficiency while reducing waste, ultimately enhancing ecological, social, and economic sustainability of aquaculture. In this study, we evaluated the feasibility of cultivating the red seaweed *Chondracanthus acicularis* in IMTA with the sea urchin *Paracentrotus lividus*.

## Materials and methods

Three experimental systems were designed and monitored over a 4-week period: (S1) a monoculture of 40 sea urchins, (S2) a co-culture of 20 sea urchins with 1 g of *C. acicularis*, and (S3) a co-culture of 40 sea urchins with 1 g of *C. acicularis*. Both the seaweed and the sea-urchins were collected in Peniche, in the centre of Portugal (39° 19' 21.8" N, 9° 21' 31.2" W). Each system consisted of an independent sump connected to three flat-bottom tanks (50 L each) used to house the sea urchins (two with 40 individuals (S1, S3), and one with 20 individuals (S2). Each sump was connected to three kreisel tanks (2.9 L capacity each). In the IMTA systems, the kreisels contained *C. acicularis* at a density of 1 $g \cdot L^{-1}$, while the control system featured empty kreisels, serving as a baseline without macroalgae. The kreisels were illuminated by white LED lamps under a 12:12 h light:dark photoperiod, with an intensity of approximately 1000 lux, and were constantly aerated.

Twice a week, physicochemical parameters including temperature, salinity, pH, ammonia, nitrites, and nitrates ($NH_3$, $NO^-_2$ and $NO^-_3$, respectively) were regularly monitored and the seaweed Relative Growth Rate (RGR, $\%.day^{-1}$) was calculated weekly to assess biomass growth.

## Results

The systems showed generally stable environmental conditions, with small variations in temperature (ranging from 18.6 to 22.3°C), pH (7.57 to 8.14), and salinity (33.84 to 37.02). Ammonia concentrations were low across all treatments, though slightly higher in the monoculture system (S1). Notably, the nitrate and nitrite concentrations in S3 were consistently higher compared to S2, suggesting enhanced nutrient availability likely derived from sea urchin excretions.

Seaweed growth data revealed a striking contrast between the two IMTA setups. In S2 (IMTA with 20 sea urchins), *C. acicularis* showed a consistent decrease in biomass, with RGR values ranging from -1.34% to -6.32% per day, indicating a net biomass loss. Conversely, in S3 (IMTA with 40 sea urchins), the seaweed exhibited positive growth throughout the experiment, with RGR values ranging from 0.32% to 1.29% per day.

## Discussion

Seaweeds are widely recognized for their high capacity to absorb dissolved nutrients, particularly nitrogen compounds, making them valuable components in IMTA systems. However, the effectiveness of seaweeds in IMTA also depends on their growth dynamics. *C. acicularis*, in particular, is known for its relatively slow growth. In a previous study, the species reached a maximum relative growth rate (RGR) of 0.945% $day^{-1}$ under optimized conditions. In the present trial, this value was surpassed in the IMTA system with 40 sea urchins (S3), which showed a peak RGR of 1.29% $day^{-1}$ during the fourth week, while the system with 20 sea urchins consistently showed negative RGR. These contrasting results suggest that nutrient input from the sea urchins through excretion was insufficient in the lower-density system to support algal growth, while the higher-density setup provided adequate dissolved nutrients to sustain and promote biomass accumulation. Thus, this study highlights the critical importance of animal stocking density and nutrient load when designing effective IMTA configurations.

# COMPOSITION AND BIOACTIVITY OF SULPHATED POLYSACCHARIDES EXTRACTED FROM *Rugulopteryx okamurae*

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## Introduction

Seaweeds are a source of bioactive compounds, including proteins, amino acids, polysaccharides fatty acids, vitamins, pigments and polyphenols with antioxidant, antimicrobial and antiviral functions. In recent years, there has been a growing interest in the research of polysaccharides of marine origin obtained from macroalgae being the most investigated for their potential applications in the fields of biomedicine and biotechnology: fucoidan, alginate and laminarin, present in brown macroalgae; ulvan obtained from green macroalgae; agar and carrageenan from red macroalgae (Salehi et al., 2019). Recently, the role of ulvan extracted from *Ulva ohnoi* as immunonutrient in *Solea senegalensis*, a species of great interest in aquaculture, has been reported (Ponce et al., 2024). Fucoidan is formed mainly by L-fucose and sulfate groups, although monosaccharides such as xylose, mannose, galactose, rhamnose and glucose, uronic acids and proteins may also be part of its structure. Like other macroalgae polysaccharides, fucoidan has a broad spectrum of biological properties, including antioxidant, immunomodulatory, antimicrobial, antiviral, anticancer and anticoagulant properties (Luthuli et al., 2019). The aim of this study was to characterize a sulphated polysaccharides extract obtained from the brown macroalgae *Rugulopteryx okamurae* and evaluate its antioxidant and immune activity. For this reason, sulphated polysaccharides extract was obtained using the hot water method and isolated by precipitation with ethanol. Chemical characterization and *in vitro* antioxidant and immunomodulatory activity was evaluated. Finally, the antimicrobial activity against *Photobacterium damselae* subsp. *piscicida*, an important fish pathogen, was determined.

## Material and methods

*R. okamurae* from Faro de Punta Carnero (Algeciras) was collected in spring. After washed, lyophilised, and milled, algal powder was mixed with ethanol 80% and stirred overnight at room temperature. The solution was centrifuged, the sediment was dried at 45ºC for 24 h and thereafter, it was treated with hot water (70ºC) for 2 h. The extract was centrifuged, and this process was repeated twice. The supernatant was collected and precipitated with ethanol overnight at 4ºC. The precipitated extract was recovered by centrifugation and freeze-dried. Total protein concentration was determined using the Bradford reagent following the manufacturer´s instructions for the 96 well plate assay. Sulfate, L-fucose, fucoidan and uronic acid content were determined as described previously by (January et al., 2019). Total phenolic assay was performed by the Folin- Ciocalteu method. For *in vitro* assays, macrophages from head kidney of 9 *S. senegalensis* juveniles obtained from facilities of IFAPA Centro El Toruño were extracted and cultured as described by Secombes and Fletcher, (1992). After 24 h of incubation with the sulphated polysaccharides extract (0.05, 0.5 y 1 mg $ml^{-1}$), cells were collected. Total RNA was isolated using the RNeasy® Mini Kit (Qiagen) and expression analyses were performed by RT-qPCR.

**Table 1.** Total phenolic compounds (TPC) and antioxidant activity

| TPC | | Antioxidant activity | | |
|---|---|---|---|---|
| Phloroglucinol (mg/g DW) | Gallic acid (mg/g DW) | DPPH (%) | $ABTS^{\bullet+}$ (µmol Trolox/g DW) | Reducing power (mg AA/ g DW) |
| 37.04 ± 0.75 | 22.96 ± 0.48 | 79.92 ± 1.31 | 352.74 ± 3.46 | 65.63 ± 1.85 |

*(Continued on next page)*

## Results and discussion

The yield of lyophilised sulphated polysaccharides extract obtained from *R. okamurae* was 3.16 ± 1.31% of the algal dry weight. High levels of L-fucose, a high sulphate content and low levels of contaminants such as uronic acid and protein are considered characteristics of good quality fucoidan (Ale et al., 2011). The sulphated polysaccharides extract obtained in this study contained 13.33 ± 2.92%, 5.34 ± 0.93% and 11.45 ± 2.14% of sulphate, L-fucose and fucoidan, respectively. Moreover, protein and uronic acid content was 2.34 ± 0.20 and 15.70 ± 0.81%, respectively. The protein content was lower than 3% indicating the purity of the sulphated polysaccharides extract. The main sugars were fucose (23.55 ± 0.03%), mannose (21.14 ± 1.0%) and xylose (15.17 ± 0.91) followed by galactose (13.78 ± 1.44%), glucuronic acid (10.19 ± 0.74%), glucose (8.58 ± 0.9) and rhamnose (8.01 ± 0.93%).

The results obtained in this study indicate that the sulphated polysaccharides extract has antioxidant activity (Table 1). The polyphenol content of macroalgae contributes significantly to their antioxidant and immunomodulatory properties. Therefore, these results could be explained by the higher content of phenolic compounds associated with the sulphated polysaccharides extract. To investigate the effects of the sulphated polysaccharides extract on the defence system, the expression level of a panel of genes related to the immune system was evaluated in Senegalese sole macrophages. The selected genes were as follows: lysozyme g (*lyg*), interleukin 1b (*il1b*), interleukin 6 (*il6*), interleukin 10 (*il10*), complement C3 (*c3*), hepcidin (*hamp*) and transferrin (*tf*). A significant coordinated increase in the mRNA levels of *il1b*, *il6*, *il10, hamp* and *tf* were observed in macrophages incubated with the sulphated polysaccharides extract at all concentrations assayed (0.05, 0.5 and 1 mg $ml^{-1}$) with respect to the control groups whereas no significant differences were observed in *c3* expression levels. All these results together with the antibacterial activity of the sulphated polysaccharides extract will be presented and discussed in depth during the oral presentation.

## Acknowledgements

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# EFFECTS OF DIETARY ULVAN ON IMMUNE GENE EXPRESSION AND DISEASE RESISTANCE IN EUROPEAN SEABASS

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## Introduction

For the first time, aquaculture surpassed fisheries in aquatic animal production with 94.4 million tonnes. Aquaculture has the potential to significantly meet the rising global demand for aquatic foods although future expansion must prioritize sustainability (FAO, 2024). Researchers are exploring alternative preventive methods to reduce reliance on antibiotics and chemotherapeutics for infectious disease control. In this way, there has been a growing interest in the research of marine-derived polysaccharides (MDPs) due to their potential to enhance the immune responses of aquatic animals (Mohan et al., 2019). Specifically, ulvan is a sulphated MDP present in the cell wall of green macroalgae of the genus *Ulva* that has immunomodulating and antioxidant activities among others (Kidgell et al., 2019). Recently, the role of ulvan extracted from *Ulva ohnoi* as immunonutrient in Senegalese sole (*Solea senegalensis*) has been reported (Ponce et al., 2024). This study highlights the importance of considering the dosage, the time of administration and the prolonged effects after a withdrawal period. However, dietary ulvan effects in European seabass (*Dicentrarchus labrax*) remain largely unexplored. The aim of this study was to evaluate the influence of different dosages of dietary ulvan on immune gene expression, disease resistance and the prolonged effects after a withdrawal period in *D. labrax*. For this reason, European seabass juveniles were fed control, 0.5 and 1% ulvan supplemented diets for 15 days followed by 60 days post withdrawal (dpw). Finally, the disease resistance against *Photobacterium damselae* subsp. *piscicida*, an important fish pathogen, was determined at 60 dpw.

## Material and methods

*Ulva ohnoi* biomass was supplied by Ulva Natura S.L. and ulvan extraction was performed as described previously by Ponce et al. (2024). Briefly, after washed, lyophilised, and milled, algal powder was treated with hot water (80ºC) for 2 h. The extract was centrifuged, and this process was repeated twice. The supernatant was collected and precipitated with ethanol overnight at 4ºC. The precipitated ulvan-rich extract was recovered by centrifugation and freeze-dried. European seabass juveniles (average weight 14.26 ± 1.55 g) were provided by Cupibar S.L. (Cádiz, Spain) and three experimental groups were stablished in triplicate: control, 0.5 and 1% ulvan supplemented diets. The feeds were developed by LifeBioencapsulation S.L. (Almería, Spain). Juveniles were fed the experimental diets for 15 days followed by 60 days of withdrawal. At 15 days post-treatment (dpt), as well as at 30 and 60 days post-withdrawal (dpw), spleen samples were collected, transferred to RNAlater™ Stabilization Solution (Invitrogen), and stored at -80°C until further analysis. Total RNA from spleen was isolated using the RNeasy® Mini Kit (Qiagen) and treated twice with DNase I using the RNase-Free DNase kit (Qiagen). Total RNA was reverse transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad). Expression analyses of a set of genes related to immune system were performed by RT-qPCR using specific primers: lysozyme g (*lyg*), interleukin 1b (*il1b*), interleukin 6 (*il6*), interleukin 10 (*il10*), tumor necrosis factor alpha (*tnfa*), hepcidin (*hamp*) and transferrin (*tf*). At 60 dpw *D. labrax* juveniles were challenged with *P. damselae* subsp. piscicida ($2\ 10^7$ cfu $g^{-1}$), and mortality was recorded each day.

## Results and discussion

To assess the effects of dietary ulvan on the immune system of European seabass, the expression profiles of seven genes were evaluated. No significant differences were observed for the expression levels of *il1b*, *il10* and *tf* in any case. When juveniles were fed the experimental diets supplemented with 0.5% ulvan, mRNA levels of *il6* and *hamp* were significantly upregulated with respect to the control groups only at 60 dpw. In the case of 1% ulvan treated groups, only expression levels of *il6* were significantly downregulated with respect to the controls at 15 dpt. Nevertheless, expression levels of *lyg*, *il6*, *tnfa* and *hamp* increased significantly with respect to the control groups at both, 30 and 60 dpw. Moreover, when juveniles were challenged with *P. damselae* subsp. *piscicida* at 60 dpw, the relative percentage survival (RPS) in juveniles from 0.5% and 1% ulvan treated

*(Continued on next page)*

groups was 31.58 and 42.11%, respectively at 7 days post challenge (dpc). Indeed, survival of 1% (54.17%) was signifcantly higher than controls (20.83%). No mortality was observed in juveniles from PBS injected groups throughtout the challenge period. Similar results were observed previously by Ponce et al., 2024 in Senegalese sole. In their study, administration of 1% dietary ulvan for 30 days resulted in an enhanced immune status and increased resistance against *P. damselae* subsp. *piscicida* and these effects were prolonged over time. Overall, these results demonstrate that dietary ulvan modulates the inmune gene expression levels and increases the disease resistance against *P. damselae* subsp. *piscicida* in *D. labrax* juveniles when is administered for 15 days at 1%. Moreover, these results highlight the importance of dosage, timing and target species for establishing a suitable protocol for the administration of immunomodulators such as ulvan in aquaculture.

**Acknowledgements**

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# SHORT-TERM CHILLED PRESERVATION OF *Litopenaeus stylirostris* SPERMATOPHORES

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## Introduction

The blue shrimp *Litopenaeus stylirostris* is naturally found on the Pacific East coast from northern Mexico to Peru. In the 1970s this species was identified as a good candidate for domestication. It was thus introduced straightaway in French Polynesia, New-Caledonia and Hawaï (Haffray et al., 2025). Nowadays, selective breeding programs are implemented in French Polynesia and New Caledonia. Manual fertilization to ensure controlled mating is common practice. To do so, the sperm mass contained in the spermatophores of the males is sampled and placed on the gonopores of the females for fertilization to occur. In order to ease that process, the aim would be to sample the desirable males whenever they are mature and store their spermatophore in the fridge, waiting for the wanted females to be about to spawn and fertilized thanks to these chilled spermatophores.

## Material and Method

The males used in this study were 10 months of age (~ 40 g) and were reared and prepared as breeders according to the practices of the commercial hatchery VAIA (Vairao, Tahiti). Only mature males with two spermatophores (non-melanized) were sampled. In total, 88 spermatophores from 88 different males were placed in a tube within a preservation solution for storage in the fridge (4°C). This solution was a saline calcium-free solution containing antibiotics, as inspired by Nimrat et al., 2020. Spermatophores were assessed for viability every day, starting the next day of the sampling and for two weeks. To do so, a suspension of spermatozoa obtained from the sperm mass from the spermatophores was stained with the pink dye eosine and observed under a light microscope. Unstained cells were considered alive whereas pink-colored cells were considered non-viable.

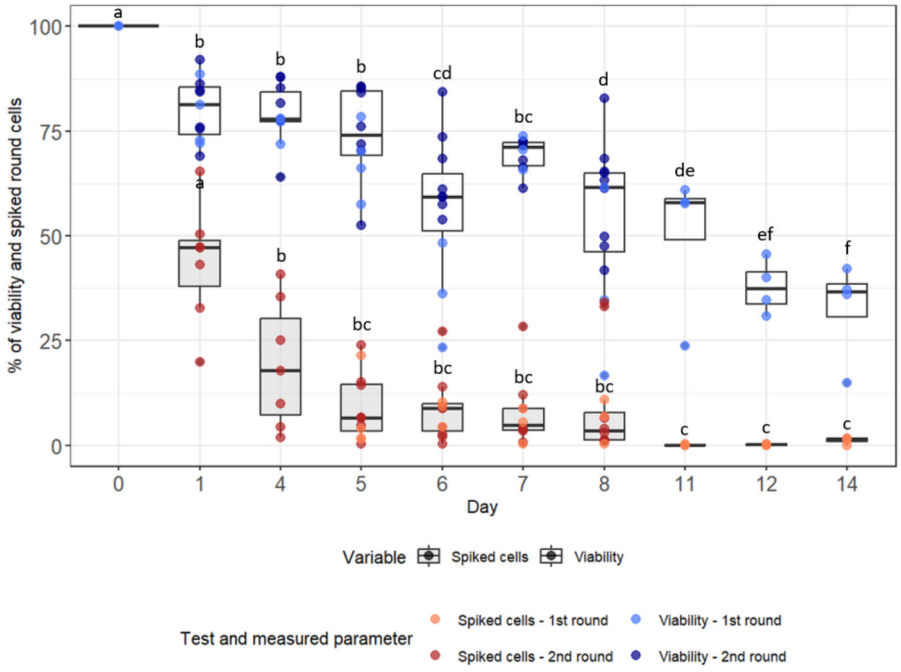


Figure 1: Assessment of sperm viability during chill storage of spermatophores

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**Results**

Statistically, as shown in Figure 1, there was a significative difference in mean viability since the first day of chilled-storage compared to the perfect full viability of the control. However, during the first 5 days, the viability remained stable above around 78% in mean. The viability dropped more noticeably after 8 days of chilled-storage and reached a mean of 37%.

**Conclusions**

Future work will aim at optimizing the current protocol to sample and preserve the spermatophore (*e.g.* composition of the solution) so that to keep viability more stable over time and at an even higher level.

As such, these results are promising to facilitate the use of *Litopenaeus stylirostris* males for artificial fertilization in hatcheries, as it provides some flexibility in the operational organization of the mating plans. It can also be a very interesting way to easily provide some male genetic resources to other hatcheries if transportation in chilled conditions does not exceed one week.

**Acknowledgements**

We feel very indebted towards the DRM who owns the shrimp line and VAIA hatchery in Tahiti financing 100% of the blue shrimp production through a public market with the CAPF: they let us use part of their broodstock in order to conduct the experiment. We warmly thank IsBlue for the mobility grant, the UPF for the training grant, OSO farming, Blue Genetics, Aquapesca and UPRA-NC Crevette for their financial support. We also thank also Ifremer (Vairao) for the place allocated to achieve the sampling of the animals.

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# FEEDING TOMORROW'S SHRIMP: UK-SOURCED, LOW-EMISSION AQUAFEEDS TO DRIVE SUSTAINABLE RAS-SECTOR GROWTH

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## Introduction

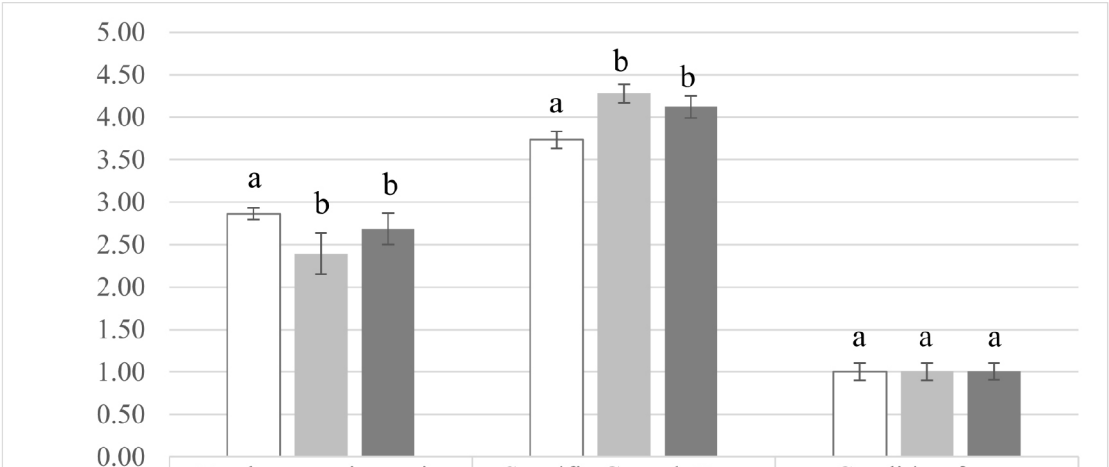
The UK shrimp recirculating aquaculture system (RAS) sector is still in its early stages of development and remains heavily dependent on imported aquafeeds. This reliance not only increases the sector's carbon footprint and feed costs but also exposes it to supply chain vulnerabilities. To address these challenges, the InSuRAFeed project was launched in 2023 with the aim of developing cost-effective, nutritionally balanced diets for Litopenaeus vannamei, formulated exclusively from UK-sourced, sustainably produced ingredients. By focusing on locally available raw materials and innovative feed formulations, the project seeks to enhance the sustainability, resilience, and economic viability of UK shrimp RAS operations.

## Methods

Twenty-two candidate ingredients were evaluated for nutrient profile, sustainability, and cost. Two experimental diets were formulated: high animal/low plant protein (HA/LP; 19 %:71 % animal-to-plant) and low animal/high plant protein (LA/HP; 13 %:76 %), both iso-nitrogenous, iso-lipidic, and iso-calorific with a commercial control. Diets were produced in-house and trialed in vivo over 50 days. Growth performance, feed conversion ratio (FCR), specific growth rate (SGR), condition factor (K), carcass composition, and apparent digestibility coefficients (ADCs) were measured.

## Results

Initial weights (1.47–1.50 g) were comparable across treatments. Final weights increased 8.15-fold (HA/LP) and 7.54-fold (LA/HP) versus 6.2-fold for the control ($p < 0.05$). SGR was significantly higher in both experimental groups; FCR was lowest for HA/LP but not significantly different from the control. K-factors (1.0–1.5) indicated optimal condition in all groups. Carcass protein (76.0–77.1% DM) was consistent, lipids slightly lower in LA/HP (4.68% DM), and ash higher in experimental diets. Protein ADCs were 78.8–83.9%, lipid 90.0–92.6%, phosphorus 96.5–97.1%, with moderate ash digestibility (36.8–44.9%). Cannibalism during molting likely affected feed metrics.




| | Feed conversion ratio | Specific Growth Rate | Condition factor |
|---|---|---|---|
| Control | 2.86 | 3.73 | 1.01 |
| HA/LP | 2.39 | 4.28 | 1.01 |
| LA/HP | 2.68 | 4.12 | 1.01 |

Figure 1. Average Feed Conversion Ratio, Specific Growth Rate, and Condition factor for *Litopenaeus vannamei* administered the different experimental aquafeeds for 50 days. Data presented as mean ± SD. Different letters above the columns denote significant differences.

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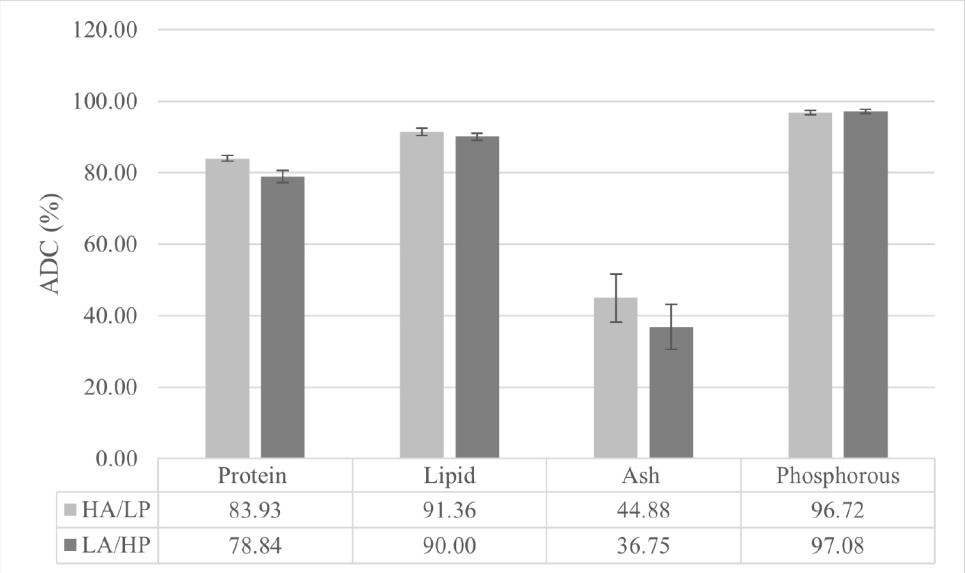


Figure 2. Apparent Digestibility Coefficients of the different experimental feeds when fed to *Litopenaeus vannamei.*

**Discussion and future work**

UK-sourced, soy-free diets can match or exceed the performance of imported feeds while maintaining product quality and nutrient digestibility. Future work will refine formulations to reduce cost, mitigate cannibalism impacts, and enhance scalability. Ongoing analyses include hepatopancreas histology, haematology, life cycle assessment (LCA), and waste particle characterisation to further optimise sustainable UK shrimp production. These findings demonstrate the feasibility of producing high-performance, UK-sourced diets for shrimp RAS without soy or imported proteins. Ongoing work includes hepatopancreas histology, haematological profiling, life cycle assessment (LCA) of ingredient sourcing, and waste characterisation for RAS optimisation. Refinement of formulations aims to reduce costs and enhance scalability, supporting sustainable UK shrimp production.

# INNOVATIVE SUSTAINABLE RECIRCULATING AQAUACULTRE SYSTEM AQUAFEEDS (InSuRAFeeds) FOR MIRROR CARP *Cyprinus carpio* UTILISING EXCLUSIVLEY UK-SOURCED, NON-SOY, LOW-EMISSION INGREDIENTS

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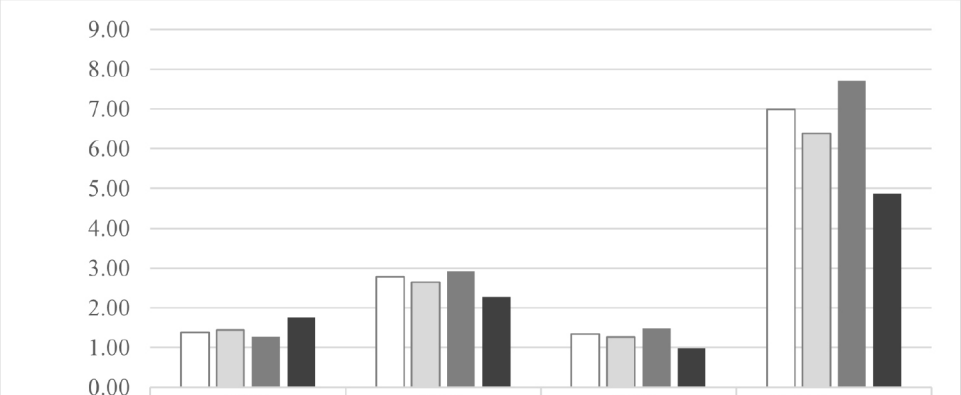
**Introduction**

Within the industry, there is a growing demand for sustainable and resource-efficient aquaculture feed, particularly for Recirculating Aquaculture Systems (RAS), while minimizing environmental impacts and ensuring healthy and sustainable diets. This research aimed to produce novel experimental RAS diet formulations, utilising non-soy, sustainably sourced, low-emission, UK-produced ingredients, exclusively, that are comparable to current commercially available aquafeeds, in terms of fish growth and nutrient digestibility.

**Methodology**

Of the 30 ingredient suppliers who were approach to participate in the project, 22 fully engaged. Following comprehensive proximate composition analysis, 3 iso-lipidic and iso-nitrogenous experimental feeds were formulated to meet the known minimum nutritional requirements of mirror carp, and manufactured in-house using a Clextral twin-screw extruder. The *in vivo* feeding trial (70-days: $BW_i = 8.64 \pm 0.02$g) was conducted in a RAS at Pontus Research's research facility, with dietary regimes assigned randomly in quadruplicate. Standard health and growth metrics (WG, % survival, FCR, SGR, and K-factor, were monitored, and faeces collected for digestibility analysis.




| | FCR | SGR | PER | Fold increase |
|---|---|---|---|---|
| Control | 1.38 | 2.77 | 1.35 | 6.98 |
| Diet 1 | 1.45 | 2.65 | 1.26 | 6.39 |
| Diet 2 | 1.28 | 2.92 | 1.48 | 7.71 |
| Diet 3 | 1.75 | 2.28 | 0.99 | 4.87 |

Figure 1. Growth parameters (FCR = Feed conversion ration, SGR = Specific Growth Rate, PER = Protein Efficiency Ratio) of mirror carp administerd the different experimental aquafeeds for 70 days.

*(Continued on next page)*

**Results**

Final weights increased 6.98-, 6.39-, 7.71- and 4.87-fold, respectively, for Control, and Diets- 1, 2 and 3. The SGR for fish administered Diet 2was significantly higher than the of the control-fed fish, which also demonstrated a significantly lower FCR.

Carcass composition and digestibility analysis, histological examination of the gills, skin and gut, haematology parameter analysis, and Life Cycle Analysis of the experimental diets all remain ongoing. We hope to have the data ready in time to present on the poster, should we be successfully chosen to present.

**Discussion**

From the results collated so far, we have demonstrated the potential for carp, raised in RAS, to be administered aquafeeds comprised exclusively of UK-sourced ingredients, decreasing industry dependency on soy and the associated carbon footprint of aquafeeds. We aim to demonstrate that diets formulated from UK-sourced, soy-free ingredients can achieve performance comparable to—or even exceeding—that of imported feeds, while maintaining high product quality and nutrient digestibility. Future research will focus on refining formulations to lower costs and improve scalability.

# DEVELOPING SUSTAINABLE, UK-SOURCED, LOW-EMISSION RAS DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

As the aquaculture sector continues to expand, there is a growing need for feeds that are not only nutritionally effective but also sustainable and resource-efficient, particularly within Recirculating Aquaculture Systems (RAS). Reducing the environmental footprint of aquafeeds while maintaining fish health and growth is a key challenge for the industry. In response, this research focused on the development of innovative RAS diet formulations made entirely from non-soy, sustainably sourced, low-emission, UK-produced ingredients. The aim was to produce diets that are comparable—or superior—to existing commercial feeds in terms of growth performance, nutrient digestibility, and feed efficiency, demonstrating a practical pathway toward locally sourced, environmentally responsible aquaculture nutrition.

## Methodology

Of the 30 ingredient suppliers approached to participate in the project, 22 fully engaged. Based on comprehensive proximate composition analysis, three iso-lipidic and iso-nitrogenous experimental feeds were formulated to meet the known minimum nutritional requirements of Rainbow trout and were manufactured in-house using a Clextral twin-screw extruder. The *in vivo* feeding trial (initial body weight = 27.64 ± 0.15 g) is being conducted at Pontus Research and remains on-going, with a scheduled end date of the 19th August.

Over the course of the 63-day trial, growth parameters and indices (FCR, SGR, WG, PER, survival and K-factor) have been closely monitored and will be presented in the conference poster. In addition, data on apparent digestibility coefficients, carcass composition, waste particle characterisation, epidermal mucous quality, and a range of haematological parameters will be included, providing a comprehensive assessment of the effects of the experimental diets on both growth performance and overall fish health. Gut, gill and skin samples will also be taken for histological examination at the conclusion of the trial but it is unclear whether these analysis will be completed in tie for inclusion in the poster.

## Significance

The InSuRAFeed project addresses a critical challenge in the UK aquaculture sector: the heavy reliance on imported feeds, which increases production costs, supply chain vulnerability, and the environmental footprint of farming operations. By developing nutritionally balanced, cost-effective diets formulated exclusively from UK-sourced, sustainably produced ingredients, the project aims to demonstrate that local feed resources can support high growth performance and fish health while reducing carbon emissions.

For the industry, the outcomes of InSuRAFeed are highly impactful. The project will provide evidence-based formulations that allow farmers to reduce dependency on imported ingredients, lower operational costs, and improve sustainability credentials. Additionally, by optimizing diet composition for recirculating aquaculture systems (RAS), the project supports efficient resource use, waste reduction, and improved animal welfare, setting a new benchmark for sustainable UK aquaculture. Ultimately, InSuRAFeed has the potential to transform industry practice, strengthen domestic feed production, and position the UK as a leader in low-emission, sustainable aquaculture.

# CENTRAL INFLAMMATORY RESPONSE IN RAINBOW TROUT AND ITS IMPLICATIONS IN APPETITE REGULATION

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## Introduction

Increased dietary fat has been used in aquafeeds to enhance fish growth while reducing costs, as fats are energy rich and cheap (Naiel *et al.*, 2023). However, in mammals, high-fat diets (HFD) seem to trigger a generalized inflammatory response that alters feed intake regulation mechanisms (Le Thuc *et al.*, 2017), leading to hyperphagia (Lee *et al.*, 2020), linking inflammation and feed intake processes. In mammals, dietary fat triggers a rapid hypothalamic inflammatory response 24 h after the first feeding of HFD and reaches a peak around the third day, descending for 2-3 weeks, and rising again becoming chronic and systemic (Thaler *et al.*, 2013; Jais y Brüning, 2017, Lee *et al.*, 2020). Mechanistically, two major inflammatory pathways are activated: those mediated by JNK and NF-κB. As a result, proinflammatory cytokines are produced (Le Thuc *et al.*, 2017). Additionally, oxidative stress seems to be impaired because of toxic lipidic species accumulation (Cavaliere *et al.*). Saturated fatty acids seem to be the trigger of this complex response (Chataigner *et al.*, 2021). In fish, little is known about the relationship of feeding with inflammatory response associated to dietary fat. The inflammatory response has been mostly studied at peripheral levels and central effects have been demonstrated in fish (Dai *et al.*, 2018; Ghaddar *et al.*, 2020). The purpose of this study was to determine if the central inflammatory response to dietary fat observed in mammals is conserved in a teleost fish and if it is also related with feed intake.

## Materials and methods

60 rainbow trout (*Oncorhynchus mykiss*) were randomly distributed into 4 equal tanks and fed a commercial diet (Biomar, Spain) during the acclimatation period. Then, two tanks were fed the control diet (CD), whereas the other two were fed the high-fat diet (HFD), with a 24 and 32 (% DM) of fat content, respectively. Fish were fed until visual satiety once a day and feed intake levels were recorded. 3 and 17 days after eating the experimental diets (Sparos, Portugal), fish were anaesthetized and euthanized to collect blood, hypothalamus and telencephalon, that were immediately frozen in dry ice and stored at -80 ºC until further analysis. Glucose, lactate, fatty acids and triglycerides concentrations were measured in plasma. The relative amount of mRNA from genes related with inflammatory response, oxidative stress and feed intake regulation in hypothalamus and telencephalon was quantified by RT-PCR. Statistical analysis was performed to all data using R and RStudio software. ANOVA, Tukey tests and non-parametric Kruskall-Wallis tests were done as needed.

## Results

HFD led to a significantly higher plasmatic fatty acid concentration. An increasing trend was also observed in plasmatic triglycerides concentration. Antiinflammatory cytokines (*tgf-β1*and *il-10*) mRNA relative abundance showed a general tendency to increase over time in both (hypothalamus and telencephalon) (***Fig. 1A-D)***. Regarding proinflammatory cytokines, *tnf-α* mRNA relative abundance increased at long term in HFD group in telencephalon, whereas in hypothalamus, there is a decreasing trend in short term (***Fig. 1E-F)***. Genes related to inflammatory pathways (*ikkα* and *ikkβ*) showed an increasing tendency at 17 days in both tissues in HFD group. All three oxidative stress related genes mRNA relative abundance showed an increasing trend in both tissues at long term (data not shown). As for feed intake regulation, hypothalamic anorexigenic neuropeptides (*pomca1, cartpt*) mRNA relative abundance tended to increase at long term in HFD. Orexigenic neuropeptides (*npy* and *agrp*) mRNA abundance showed an increasing trend in those animals fed the HFD at short term in the hypothalamus (data not shown).

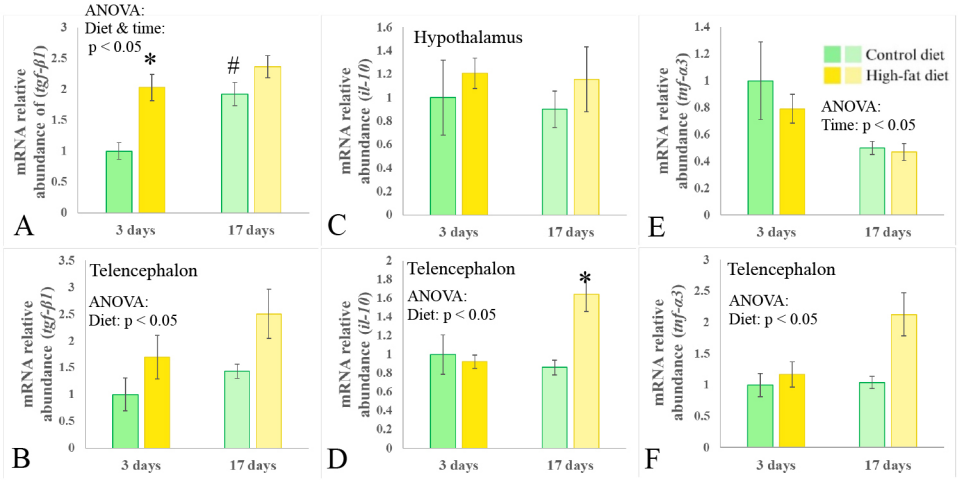


***Figure 1***.-mRNA relative abundance for 3 of the measured genes, in hypothalamus and telencephalon from rainbow trout after feeding with control or high fat diet at short and long term. A: *tgf-β1* in hypothalamus. B: *tgf-β1* in telencephalon. C: *il-10* in hypothalamus. D: *il-10* in telencephalon. E: *tnf-α3* in hypothalamus. F: *tnf-α3* in telencephalon. Bars are mean ± standard error of mean (n = 5-6). Results were analysed using ANOVA and Tukey test for post-hoc comparisons. *: significantly different from the group fed control diet at the same time ($p < 0.05$). #: significantly different from the group fed the same diet at 3 days ($p < 0.05$).

***Figure 1***.-mRNA relative abundance for 3 of the measured genes, in hypothalamus and telencephalon from rainbow trout after feeding with control or high fat diet at short and long term. A: *tgf-β1* in hypothalamus. B: *tgf-β1* in telencephalon. C: *il-10* in hypothalamus. D: *il-10* in telencephalon. E: *tnf-α3* in hypothalamus. F: *tnf-α3* in telencephalon. Bars are mean ± standard error of mean (n = 5-6). Results were analysed using ANOVA and Tukey test for post-hoc comparisons. *: significantly different from the group fed control diet at the same time ($p < 0.05$). #: significantly different from the group fed the same diet at 3 days ($p < 0.05$).

**Discussion and conclusions**

Inflammatory response seemed to be stronger and more consistent at telencephalon level attending to mRNA relative abundance variations. The increasing tendency observed in genes involved in inflammatory pathways along with the changes observed in pro- and anti-inflammatory genes support the hypothesis of a conserved inflammatory response to HFD at central level in teleost fish as observed previously in mammals (Souza *et al.*, 2016; Cavaliere *et al.*, 2018). Additionally, the pattern of variation displayed suggests that the sampling times, selected in accordance with the pattern observed in mammals, were not the optimal to catch the peak of inflammation, that could be due to immune responses being slower in fish (Watts *et al.*, 2001). On the other hand, a long-term orexigenic response was observed.

In summary, results suggest the existence of an inflammatory response against high fat diets that develops slower than in mammals. Moreover, this response seems to be related with homeostatic regulation of feeding, and could also be related to hedonic control, since telencephalon is the main brain area affected. Further studies are needed to address both the implication of hedonic control and the temporal pattern of inflammatory response.

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# EVALUATION OF THE WELFARE OF RAINBOW TROUT (*Oncorhynchus mykiss*) IN AQUACULTURE: IDENTIFICATION AND VALIDATION OF RELEVANT HEALTH AND BEHAVIORAL INDICATORS

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**Introduction**

Supported by the evolution of scientific knowledge and by a growing societal sensitivity towards animal welfare, the welfare of farmed fish is today a major issue. However, its assessment remains difficult due to the biological particularities specific to each aquatic species and the specificities of their environment. The indicators usually used for terrestrial animals are not directly transposable to fish.

In this context, a study was carried out by the consulting firm Bankiva, at the request of a French retailer, in order to identify and validate relevant indicators to assess the welfare of rainbow trout (*Oncorhynchus mykiss*) farmed in aquaculture.

The protocol implemented was based on the evaluation of thirteen health indicators (related to the state of health) and twelve behavioral indicators. Each indicator was examined with regard to five validation criteria: prevalence (Number of fish affected by a disease or expressing certain behaviors. The indicator must be observed frequently enough to validate this criterion.), specificity (Ensures that the indicator measures what we want to evaluate and not another factor. Specificity can be evaluated by taking two situations, one where the level of welfare is good and one where the level of welfare is degraded.), feasibility (Property allowing an indicator to be used in a farming context in the greatest possible number of situations and by the greatest possible number of observers.), sensitivity (Property of an indicator that makes it possible to detect early variations in welfare. Thus an indicator that only shows a variation in welfare very late or as a last resort would not be considered relevant.) and reproducibility (This consists of testing whether the same results are obtained after observing the same situation (here the same tank) by two different people.).

**Materials and methods**

The study was carried out on a large sample: 22,230 trout distributed in 18 tanks from 5 different fish farms, with varied practices (stocking densities, water recirculation systems, feeding strategies). The observations concerned three categories:

- Portion trout (250 g)
- Large trout (1–2 kg)
- Very large trout (2–3 kg)

Health observations were carried out individually on all the fish. Behavioral observations, on the other hand, focused on 54 groups (three areas per tank: head, middle, and tail). The indicators were observed according to a specific protocol:

- For behavior, visual scans every 5 minutes.
- For health, samples of 30 fish per area, observed directly in the water.

In total, 13 health indicators and 12 behavioral indicators were evaluated in each area. Their validation was based on:

- Prevalence: frequency of observation sufficient to be useful.
- Specificity: ability to measure a specific aspect of welfare, independently of other factors.
- Feasibility: applicability in the field, in different contexts.
- Sensitivity: early detection of variations in welfare.
- Reproducibility: consistency of results between observers.

*(Continued on next page)*

**Results**

The first results show that eight health indicators and six behavioral indicators meet all the validation criteria and can therefore be considered reliable for assessing the welfare of rainbow trout in aquaculture. However, five health indicators and six behavioral indicators did not meet all the criteria, revealing the difficulty of identifying robust and universal tools in fish farming.

Some non-validated indicators (e.g., vertebral deformities, hyperventilation) are rare but may indicate serious problems and act as sentinel indicators. Others, such as fin erosion, vary according to the physiological stage, suggesting a differentiated application. Observer training proved essential to improve reproducibility, notably through the use of standardized scoring grids.

**Conclusion**

In conclusion, this exploratory study highlights the complexity of selecting indicators and the challenges related to their practical application under production conditions. It paves the way for the development of protocols better suited to the specific needs of the species and to the realities of aquaculture farms. It also emphasizes the importance of continuing research to strengthen and refine fish welfare assessment tools, with the aim of continuously improving farming practices.

# SELECTIVE BREEDING OF AMUR MIRROR CARP: GENETIC VARIATION, CURRENT STATUS AND FUTURE CHALLENGES

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## Introduction

Common carp is the dominant fish species in the Czech pond aquaculture, but no systematic selective breeding program has been realised until recently. The Amur mirror carp (AMC), a new Czech mirror carp breed established in 2014, has been used as a model fish species for a long-term study to estimate the genetic, practical, ecological and economical potential of using common carp for selective breeding under traditional pond aquaculture (Prchal et al., 2018, 2021, 2024; Kocour et al., 2025). Across various studies, different genetic parameters of performance and morphological traits were estimated from the yearlings to the market size fish. Moreover, attempts to shorten the generation interval in selective breeding program of common carp were performed, genetic markers for an effective pedigree reconstruction were designed and tested and the economic and ecological impacts of genetically improved carp stocks cultured under traditional pond aquaculture were evaluated to obtain comprehensive information for assessment of effectivity, sustainability and feasibility of selective breeding program in common carp under semi-intensive pond culture management.

## Materials and methods

For most studies, AMC stocks were used. The stocks were established by a partial factorial design to obtain up to 200 full-sib families within each study. All stocks were always reared communally under semi-intensive pond conditions or in combination with a RAS system to shorten the generation interval. The parentage assignment was done using microsatellite markers (Prchal et al., 2018) or SNP markers identified by RAD sequencing (Palaiokostas et al., 2018a). The fish were phenotyped after each growing and winter period until market size. The main performance traits were, for example, body weight, Fulton's condition factor, morphological indicators, muscle fat content, slaughter yields and their predictors, scale pattern quality or flesh colour traits (CIE System). The genetic and genomic parameters were estimated using pedigree or genomic markers using an animal model and relevant fixed effects. GWASs have also been performed to study the potential of using marker-assisted selection for KHV resistance and juvenile growth (Palaiokostas et al., 2018ab). The effect of stocks with higher performance potential on production and water quality parameters in fishponds was evaluated using two stocks (M2 represented the standard population, and M2 x AMC represented the one with higher performance potential) with an internal control group. The selective breeding program in AMC was established in 2017 using a phenotypic selection on growth with a 10% selection pressure and suitable scaly patterns of the mirror scaliness phenotype. The breeding population has been reared till two years old in a fishpond and then transferred into the RAS to reach earlier sex maturation. In 2020, the first successful spawning of selected and early-matured broodstock was realised, and in 2023, so far, the last selected population (AMC-S3) has been established.

## Results

Mostly high heritability estimates were found for the main traits in common carp such as muscle fat content (0.62-0.67), morphological traits (0.93-0.98), flesh colour traits (0.16-0.39), slaughter yields (0.46-0.50) and their predictors (0.40-0.41), and for scaly patterns quality (0.62-0.89). The body weight showed high heritability both at the age of two years (0.51) and at market size (0.63). In addition, a high genetic correlation (0.74) between these age stages has been found. No undesirable genetic correlations were found in the case of selection for higher performance. Although no strong QTLs for KHV resistance and juvenile growth were found, genomic selection, even using a low-density SNP panel, was shown to be more accurate than one based on pedigree information. Based on the RAD-seq results, 270 SNPs are now used for parentage assignment in AMC (~90% assignment success). A pilot study of rearing AMC broodstock in a combination of fishpond and RAS showed the possibility of shortening the generation interval in the selective breeding program from five to three years. The selection challenge may be performed after two years of fishpond rearing, and the selected individuals are then reared at ~20 °C in RAS. The carp stock with higher performance potential achieved significantly higher mass yield by up to 59% than standard carp stocks (Prchal et al., 2024). In addition, no effect of higher mass yields of common carp on water quality parameters in fishponds was observed (Kocour et al., 2025).

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**Conclusion and future challenges**

Results indicate that AMC displays very good heritability estimates for the most important performance traits. Still, the ongoing breeding program only focuses on the rapid growth and standard mirror scaly phenotype using phenotypic mass selection. Incorporation of i) other important traits (e.g. slaughter yields, resistance), ii) using a multi-trait selection index method, iii) selection based on the estimated breeding values using pedigree or genomic information into the selection process are future challenges. On the other hand, the current breeding strategy in AMC is based on a three-year cycle to speed up the cumulation of genetic gain. We are unaware of any similar common carp selective breeding program in temperate climatic conditions. Also, there is a hypothetical possibility of shortening the generation interval to two years, and the realised heritability of AMC populations in fishponds shall be verified in a broad-scale performance test.

**Acknowledgements**

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# STURGEON GERM PLASM SEPARATED FROM OTHER GERM LINEAGES AS A UNIQUE MODEL FOR STUDYING PRIMORDIAL GERM CELLS IN FISH

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## Introduction

Primordial germ cells (PGCs) in fish are typically specified through maternally inherited germ plasm. In sturgeons (Acipenseridae), this localization is spatially and developmentally distinct, resulting in a unique natural separation of germ cells from somatic lineages during early embryogenesis. Unlike most fish species, sturgeons exhibit holoblastic cleavage and an embryonic development pattern that more closely resembles amphibians rather than typical teleost fishes. This distinctive embryonic architecture establishes sturgeon embryos as a powerful model for studying PGC biology, mitochondrial contributions to germline development, and evolutionary transitions in cleavage patterns.

## Methods

We combined PGC visualization using FITC-dextran and GFP-nos3 3'UTR mRNA microinjections, targeted UV irradiation to eliminate vegetal germ plasm, and mitochondrial transplantation experiments in different sturgeon species. Recent findings on the role of vegetal blastomeres and novel techniques for selective blastomere inhibition were integrated. Histological and gene expression analyses (qPCR tomography) were performed to distinguish embryonic and extra-embryonic contributions.

## Results

Our data confirmed that sturgeon vegetal blastomeres primarily contribute to PGC formation, while the majority differentiate into transcriptionally inactive yolk cells serving as an extra-embryonic nutritional source. UV irradiation of the vegetal pole effectively ablated PGCs without disrupting somatic development, verifying the independence of early somatic patterning from germ plasm. Mitochondrial transplantation successfully restored PGC formation even when endogenous germ plasm was destroyed, suggesting mitochondria contribute critically to germline specification. Fate-mapping studies indicated that vegetal yolk cells do not integrate into embryonic tissues, supporting a model where sturgeons exhibit an evolutionary transition between holoblastic and meroblastic cleavage.

## Conclusion

Sturgeon embryos, with their naturally separated germ plasm and somatic territories, provide an exceptional in vivo system to study fundamental questions of germline development, mitochondrial function, and evolutionary developmental biology in fish. These insights not only advance basic understanding but also have implications for biotechnological applications, including germline conservation strategies for endangered sturgeon species.

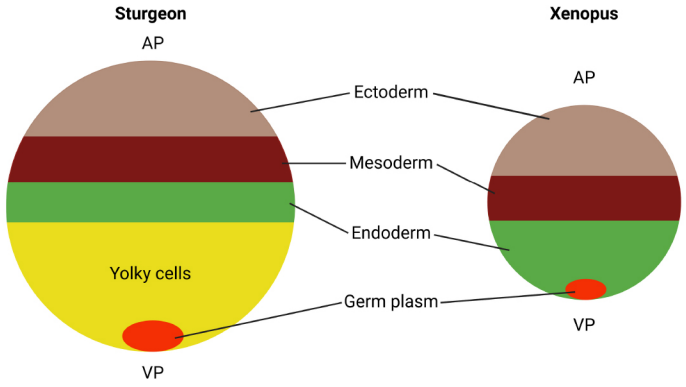


Fig 1. Comparison of germline and germ plasm determinant deposition between sturgeon and xenopus eggs (AP – animal pole, VP – vegetal pole).

# *IN VITRO* FERTILISATION OF NATURALLY OVULATED SENEGALESE SOLE (SOLEA SENEGALENSIS) EGGS FOR ORGANIC AQUACULTURE THAT INCORPORATES COMPUTER VISION PREDICTION MODELS

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**Introduction**

Senegalese sole (*Solea senegalensis*) is a valuable aquaculture species, which has reproductive challenges in captivity that hinder large-scale production (APROMAR, 2022; Morais et al., 2016). The reproductive challenges are linked to the absence of natural spawning in cultured Senegalese sole (hatched and raised in captivity) and the only method to obtain viable eggs is through *in vitro* fertilization (IVF). IVF circumvents the limitations of natural spawning by ensuring viable gametes are obtained for fertilization (Ramos-Júdez et al., 2021). However, these IVF protocols require the use of hormones to program the ovulation of viable eggs. The use of hormones in organic aquaculture is not permitted (European Commission, 2021) and, therefore, organic aquaculture necessitates hormone-free methods for predicting natural ovulation. Recent advancements in machine learning offer tools to analyse fish behaviour, potentially enabling non-invasive prediction of reproductive readiness (Zhao et al., 2021). This study aims to demonstrate the feasibility of hormone-free IVF using naturally ovulated eggs and to develop a machine learning model for predicting ovulation based on specific behavioural cues. This approach seeks to provide a sustainable and ethical alternative for enhancing reproductive outcomes for organic Senegalese sole aquaculture.

**Material and Methods**

Experimental data were collected at IRTA La Ràpita from March to May 2024, using two groups of Senegalese sole (Mix1 and Mix2) that combined wild and cultured fish. The two groups were held in 10 m³ recirculating aquaculture tanks under natural temperature and photoperiod, with specific temperature adjustments during spawning periods. Naturally spawned eggs were collected daily and assessed for viability and fertilization. For hormone-free IVF, gametes were collected between 14:00 and 24:00. Sperm was obtained via abdominal palpation, diluted with an extender (modified Leibovitz, Sigma, Spain), and assessed for quality using a Computer-Assisted Sperm Analysis (CASA) system (Microoptics, Barcelona, Spain). Females were checked for natural ovulation, and eggs were stripped for *in vitro* fertilization. Standard IVF protocols were followed using selected high-quality sperm and naturally ovulated eggs (Ramos-Júdez et al., 2021). Fertilized eggs were incubated at 16°C.

Reproductive behaviours, rest the head (RTH - an individual rests it's head on another sole) and Locomotor Activity (LA – movements that lasted more than 3 seconds) were monitored continuously using underwater aquaculture cameras. These behaviours, indicative of courtship and spawning readiness, served as inputs for the ovulation prediction model. Video footage was processed to classify behaviours using a trained behaviour detector. Fish tracking algorithms maintained individual fish IDs and calculated LA. The logistic regression model was developed and used to predict the probability of ovulation in Senegalese sole, by using RTH, LA counts and the time of behaviour occurrence as key inputs.

$$P(\text{Ovulation}) = \frac{1}{1+e^{-(\beta 0+\beta 1\cdot \text{RTH}+\beta 2\cdot \text{Locomotor Activity}+\beta 3\cdot \text{Time})}}$$

*Equation 1 Logistic regression model for predicting the probability of ovulation*

**Results and Discussion**

During the three-month spawning period, both experimental groups showed limited natural fertilization success, averaging 10-15% of all the spawns obtained. This highlights the challenges of natural spawning in captive Senegalese sole, reinforcing the need for controlled fertilization methods. In contrast, hormone-free IVF trials achieved up to 44% fertilization and 18% hatching, confirming the technical feasibility of this approach. Good quality sperm with high concentration of motile sperm per egg were used to ensure that sperm was not a limiting factor in the IVF protocol. Male gametes with superior motility (69.06 ± 13.24%), compared to low-quality sperm (39.52 ± 17.00% and 38.31 ± 13.79%, respectively) were selected and used for the IVF. This ensured fertilisation of eggs from females that had recently ovulated viable eggs to demonstrate that successful IVF of naturally ovulated eggs was possible and further highlighted the need to identify the time of natural ovulation to increase the efficacy.

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To support hormone-free IVF, an automated model was developed to detect and quantify key reproductive behaviours RTH and LA associated with ovulation. By classifying and tracking these behaviours, the system enabled the extraction of meaningful behavioural patterns that served as inputs to a logistic regression model for ovulation prediction. The integration of RTH and LA data, along with the time of behaviour occurrence, into a logistic regression model allowed for accurate prediction of ovulation timing, achieving 85% overall accuracy. The model scored 0.95 on the Area Under the Curve (AUC), a measure based on the Receiver Operating Characteristic (ROC) curve that indicates how well it distinguishes ovulatory from non-ovulatory nights. An AUC close to 1.0 means the model reliably separates these events across different thresholds, showing strong overall performance. The model correctly identified 17 out of 22 ovulatory nights and all 11 non-ovulatory nights in the test dataset, highlighting its strong performance in distinguishing ovulatory events. This suggests that non-invasive behavioural monitoring, combined with machine learning, can effectively replace hormone-induced ovulation, aligning with organic aquaculture principles. This research, for the first time, investigates that natural ovulation can produce fertilized eggs in Senegalese sole without the use of hormones, thereby pioneering a foundational concept for a more sustainable and ethical approach to control cultured sole reproduction. By strategically integrating targeted male selection for *in vitro* fertilization with an innovative automated behaviour-based ovulation prediction model, the reliance on hormonal treatments can be removed for organic aquaculture.

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**Acknowledgments**

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# A FUNCTIONAL RETROPOSON ENCODING DOUBLESEX (DSX) MEDIATES MALE SEXUAL DEVELOPMENT BY DIRECT INDUCING IAG IN THE PRAWN *Macrobrachium rosenbergii*

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The giant freshwater prawn (*Macrobrachium rosenbergii*) is an important decapod species that is widely cultured in Asia and the western Pacific. Notably, the males grow faster to reach commercial size than the females and all-male cultures produce greater mean sizes, yields, and net benefits for production of the prawn. It is well known that the male sexual development is under direct control of the insulin-like androgenic gland hormone (IAG) in decapod crustaceans. However, the regulatory mechanism of IAG has remained a mystery for several decades. Here, we identified a novel *Dsx* (*MrDsx-a*) from the prawn *M. rosenbergii*. Unlike insect *Dsx* expressed via sex-specific alternative splicing in sexual development, *MrDsx-a* is a retrogene without intron and displayed male-specific expression in the testis and androgenic gland (Fig.1). RNAi-mediated knockdown of the *MrDsx-a* can significantly down-regulate *MrIAG* in the post-larvae and ultimately resulted in male-to-female sex reversal (Fig.2). The ZZ neofemales were capable of mating with normal ZZ males and producing all male progeny. The direct interaction between the MrDsx-a protein and *MrIAG* promoter was further confirmed by ChIP-qPCR analysis and dual-luciferase reporter assays. Together, we first demonstrated that *MrDsx-a* is a sex-determining gene mediating male sexual development by direct inducing the *MrIAG* in *M. rosenbergii*, which will provide an innovative way for mono-sex breeding in the prawn aquaculture.

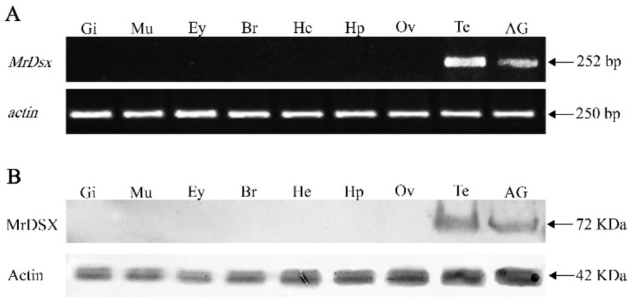


Figure 1 (A) RT-PCR (left) and Western blotting analysis (right) revealed the male-specific expression of *MrDsx-a* mRNA and protein in the adults, respectively. Marker, M; gill, Gi; muscle. Mu; eye, Ey; brain, Br; heart, He; hepatopancreas, Hp; ovary, Ov; testis, Te; androgenic gland, AG;

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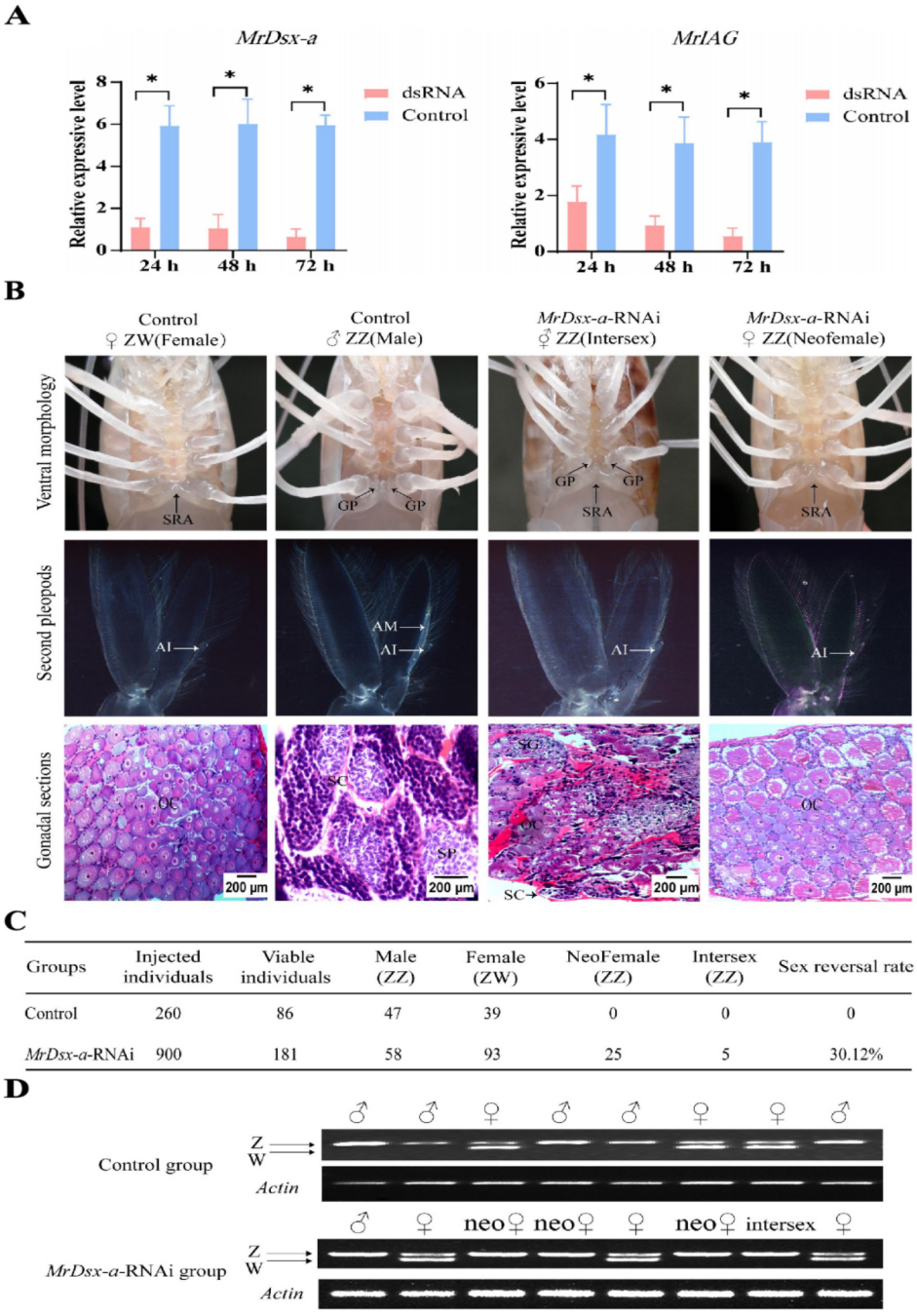


Figure 2 Effects of *MrDsx-a* RNAi in the juveniles. (A) qRT-PCR analysis of the relative expression level of *MrDsx-a* and *MrIAG* mRNAs at 24h, 48h and 72h post-injection of *MrDsx-a*-dsRNA in the juvenile males at PL40; (B) Comparisons of external sexual characteristics and histological structure of gonads of the control females and males, intersex and neofemales in RNAi groups; (B) Statistics of sex reversal individuals based on morphological observation and genetic sex identification using ZW-specific markers. (C) PCR-based sex identification on partial samples in control and *MrDsx-a* RNAi groups. AM, appendix masculine; AI, appendix interna; SRA, sperm-receiving area, GP, genital papillae. OC, oocytes. SC, spermatocyte; SG, spermatogonium, SP, spermatozoa.

# GUT HEALTH AND DIGESTIBILITY IN FARMED ATLANTIC COD *(Gadus morhua)* FED BY-PRODUCT SHELL MEALS

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## Introduction

In recent years, commercial farming of Atlantic cod (*Gadus morhua*) has resumed in Norway. However, the industry continues to face significant challenges related to fish welfare and economic losses, primarily due to intestinal disorders recently identified as strangulating obstruction (Skedsmo et al., 2024). Contemporary cod diets still rely heavily on marine-derived ingredients, in contrast to the plant-based formulations used in salmonid aquaculture, despite the growing global scarcity of marine resources. The natural diet of Atlantic cod is rich in chitin-containing organisms such as crustaceans and echinoderms, including crabs and shrimps (Lall and Nanton, 2002). This suggests that marine shell meals may represent promising side-stream candidates for inclusion in cod feeds (Tibbetts et al., 2006). Given the critical role of gastrointestinal function in overall health and nutrient absorption, a deeper understanding of cod gut physiology is necessary to identify the underlying causes of intestinal pathologies. In this context, dietary chitin has previously been reported to exert immune- and microbial modulatory effects with positive outcomes for fish gut health. The primary objective of this study was to enhance our knowledge of the Atlantic cod gastrointestinal system by evaluating two experimental diets incorporating crab and shrimp shell meals, assessing fish gut health and the digestibility of the formulated diets.

## Materials and methods

The present study evaluated the nutritional digestibility and gastrointestinal health and function of Atlantic cod with an average body weight of 300 g, fed isoenergetic diets incorporating alternative feed ingredients. Three experimental diets were formulated to be isoenergetic (20–21 kJ/g) with a digestible protein-to-energy ratio of 2.5 and approximately 50% crude protein and 17% lipid content. The control diet included 15% sunflower meal, while the other two substituted this fraction with 15% crab shell meal or 10% shrimp shell meal. Following a 27-day feeding period, fish were sampled for growth performance evaluation. Individual body weight and length, liver weight and pooled intestinal contents were recorded to determine dry matter and amino acid digestibility, and growth performance indices calculated. An additional subset of six fish per diet group was sampled for comprehensive physiological assessments. These included macroscopic examination of the digestive system in the abdominal cavity, histological analysis of intestinal tissue, gene expression profiling of the gastrointestinal tract (focusing on immunological, osmoregulatory and digestive markers), measurement of intestinal length and organ indices, bile salt and digestive enzyme assays and plasma biomarker analysis. The entire digestive system was dissected, and the intestine was sectioned into distinct anatomical regions: stomach, pyloric region with caeca, midgut (subdivided into three segments) and distal intestine. Statistical analyses were performed to assess the effects of dietary treatments and differences between anatomical segment differences, with significance determined at $p < 0.05$.

## Results and discussion

In addition to assessing digestive capacity and morphological characteristics, this study provided new insights into the spatial expression of gut-associated gene transcripts related to immune response, osmoregulation and digestion along the gastrointestinal tract of farmed Atlantic cod. A positive correlation was observed between fish body size and relative intestinal length, which may have implications for the development of digestive complications later in life. Fish fed the shrimp shell meal diet exhibited a tendency toward increased feed intake during the initial phase of the trial, resulting in a higher feed conversion ratio compared to the other dietary groups, despite overall comparable growth rates and total feed intake. The group fed crab

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shell meal demonstrated significantly higher amino acid digestibility than the other groups. However, elevated ash and calcium levels in the shell meal were associated with increased faecal output and reduced dry matter digestibility, in line with findings by Albrektsen et al. (2009). Biochemical analyses revealed elevated levels of maltase activity and bile salts in the pyloric caeca of fish fed the crab meal diet compared to the control group. No significant differences were observed among diet groups in terms of mortality or blood biomarkers associated with nutrient metabolism and only one case of strangulating obstruction occurred during the trial. Histological examination revealed increased lamina propria cellularity in the distal intestine of fish fed the control diet containing 15% sunflower meal, suggesting a potential adverse effect of sunflower meal not observed in the shell meal diets. Overall, the intestinal health of the experimental fish was assessed as normal, functional and free of significant pathological alterations.

**Conclusions**

This study provided new insights into the mapping of functional gut gene transcripts in relation to intestinal morphology and digestive capacity, serving as a reference for future research addressing intestinal strangulation in farmed Atlantic cod. Although further investigation is needed to determine whether this condition is genetically determined, diet-induced or specific to farmed populations, the present findings are essential for improving our understanding of normal gastrointestinal structure and function in this species. Shell meal from marine raw materials represents a side stream that can be used as ingredients in cod feed, helping to reduce reliance on fish meal from wild stocks and contribute to increased sustainable use of local resources.

**Acknowledgements**

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# SOCIAL PERCEPTION AND ACCEPTABILITY OF RAINBOW TROUT (*Oncorhynchus mykiss*) IN RURAL INLAND AND IN ADRIATIC AREAS, ITALY – A CASE STUDY OF CITIZENS SCIENCE

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## Introduction

The seismic events that struck central Italy in 2016–2017 had a severe impact on inland areas of the 'Alto Maceratese' in central-northern Italy (the Marche region), leading to significant economic setbacks, particularly in the local agri-food sectors (Aleffi & Cavicchi, 2020; Dottori, 2024). In order to revitalise the area, various initiatives promoting a sustainable circular economy have been implemented. In this context, the European AgrI-fiSh project (financed by PRIMA and available at agrifish.unicam.it) was conducted to recover agricultural waste. Grape by-products, such as grape seeds and pomace, can be used to create a new, high-quality, resilient aquaculture (Quagliardi et al., 2024). Two interconnected case studies were conducted in this context, involving the administration of two sets of questionnaires: a 12-question survey was given to 30 restaurant chefs, and an ongoing 4-question survey was given to consumers. Both surveys were conducted in popular tourist destinations in the Marche region, including coastal areas such as Senigallia and San Benedetto, as well as rural areas like Macerata. The aim was to assess local restaurant chefs' awareness of incorporating a regional fish species (rainbow trout) into their winter menus and to gain a better understanding of consumers' current and future perception of such species in their diets.

## Materials and Methods

The 12-question Survey Questions Administered to Restaurateurs:

1) What is the average quantity (in kilograms) of fish you purchase on a weekly basis during winter time?
2) What is the estimated total quantity (in kilograms) of fish you purchase annually?
3) Do you currently use salmon in your restaurant's menu offerings?
4) How do you typically serve salmon?
5) When purchasing salmon, do you consistently consider its origin and farming method or do you prioritize lower prices?
6) Have you ever had concerns or reservations regarding salmon farming practices?
7) Have you observed any seasonal variations in the consumption of salmon by your customers?
8) Have you ever included trout in your menu offerings?
9) Would you be open to offering trout to your customers in the future?
10) If informed that the trout is locally farmed in the Marche region, produced at near-zero food miles and without the use of antibiotics, would you consider adding it to your menu?
11) Are you aware that trout is among the most commonly consumed fish species in Northern Europe and that it can contribute to tourism-related culinary interest?
12) Based on available data indicating that trout is of higher quality and lower cost than comparable fish species, do you consider it a viable and sustainable alternative for your restaurant?

The results were collected and presented descriptively via percentages and Microsoft Excel graphs. The 4-question survey, divided by 4 age groups (0–20, 21–40, 41–60, >60), is the result of the capitalization of the first survey and has been expanded according to the scope of the AgrI-fiSh project.

## Results and discussion

During the winter period, the surveyed restaurateurs (n=29) purchased an average of 33.6 kg of fish per week, equating to 1.4 tonnes per year. However, one restaurant was excluded from the survey as it does not serve fish dishes. Nevertheless, freshwater fish remained largely absent from their menus, with 81.8% of respondents indicating that they had never served species such as rainbow trout. While 75.9% of respondents used salmon, preparation methods varied (40.9% marinated), as did awareness of sustainable sourcing. Notably, a significant proportion (77.3%) expressed interest in high-quality products, and 72.7%

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paid attention to breeding practices. Despite having limited prior experience with trout, 77.3% of restaurateurs were open to experimenting with it, though 22.7% remained sceptical even when its sustainable attributes were considered. Therefore, there is an opportunity to bridge the gap between supply and demand by informing restaurant operators about consumer expectations and the benefits of locally produced trout as an ideal seasonal alternative to commonly used species such as salmon. When comparing salmon and trout, younger participants tended to favour salmon due to taste and familiarity, while older respondents were more positive about trout, often due to habit or local availability. Many participants said they would eat more trout if it were more widely available, suggesting there is potential for increased acceptance through improved distribution. Regarding the use of by-products in trout feed, in line with the scope of the AgrI-fiSh project, most respondents were open to it or conditionally accepted it, depending on the type of by-product used. Those people aged 21–60 were most curious and open to innovation, while rejection was more frequent among the youngest group and may be linked to unfamiliarity or lack of information. In terms of sustainability, the majority of participants considered vegetable by-products to be more environmentally friendly than fish by-products, although many recognised the value of both. This indicates a growing awareness of circular practices and an informed, balanced approach to environmental choices. Overall, consumer attitudes reflected a combination of traditional values and a willingness to embrace sustainable innovations. However, differences across age groups suggest opportunities for targeted communication and education.

**Conclusions**

A revitalisation strategy that integrates the rainbow trout farming sector into coastal and inland gastronomy in central Italy seems promising. Substituting imported fish for rainbow trout in winter seafood dishes would encourage inland trout farmers to form a regional consortium and promote the highly nutritious and sustainable nature of the species. This strategy could ensure a consistent supply and maintain quality standards, as well as improving marketing efforts, particularly if a sustainable approach is adopted. This will contribute to the economic recovery of rural areas and align with contemporary food movements that emphasise local sourcing, biodiversity and environmental responsibility.

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# EFFECT OF FISH MEAL SUBSTITUTION ON GROWTH PERFORMANCE AND INTESTINAL MICROBIOTE IN *Chelon labrosus*

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## Introduction

Nowadays, the aquaculture industry faces the challenge of reducing its dependence on fish oil and fishmeal. Therefore, it is of paramount importance to develop successful innovative aquafeeds which may contribute to achieving truly sustainable rearing practices. On such account, omnivorous low trophic level species like *Chelon labrosus* have been proved to provide optimal performance results when fed with alternative land-based protein diets (Sanz Latorre, 2024). What is more, novel diets have been also evidenced to influence the fish gut microbial community and its functionality, improving the fish nutrient utilization and enhancing their immune response (Agboola et al., 2023). For this reason, this research aims to formulate and test the impact of three alternative experimental diets on *C.labrosus* growth, microbial biodiversity and its functional role.

## Materials and methods

Three isolipidic and isoenergetic diets were formulated using alternative ingredients and containing a 40% protein inclusion. The first one was 100% plant-based (V), the second one was a mix of vegetal and animal protein (VA) and the last one had a mixture of fishmeal and plant protein (FM). Then, 300 *Chelon labrosus* were kept in triplicate tanks and subjected to a 153 day trial phase in which they were administered the experimental diets. Zootechnical parameters were measured every 30 days. At the end of the experiment, 60 fish were sacrificed to obtain gastrointestinal tract samples for microbiome analysis. Composition and structure of the microbial community was assessed through the amplification and sequencing of the 16S rRNA gene. The bioinformatic analysis of the microbial metagenome was conducted using the Integrated Microbial Genome database, and the microbial functional annotation was based on the KEGG and COGs databases.

Table 1: Growth performance and nutritional parameters of *Chelon labrosus* with experimental protein sources

| | V | VA | FM |
|---|---|---|---|
| **Initial weight (g)** | $71.9^{c} \pm 0.26$ | $72.4^{ab} \pm 0.25$ | $73.2^{a} \pm 0.28$ |
| **Final weight (g)** | $104.1^{a} \pm 1.20$ | $73.8^{c} \pm 1.21$ | $92.1^{b} \pm 0.92$ |
| **SGR (% day$^{-1}$)** | $0.24^{a} \pm 0.008$ | $0.01^{c} \pm 0.009$ | $0.15^{b} \pm 0.005$ |
| **Weight gain (%)** | $144.9^{a} \pm 1.05$ | $101.9^{c} \pm 1.44$ | $125.8^{b} \pm 1.04$ |
| **FCR** | $4.58^{a} \pm 0.067$ | $13.12^{c} \pm 2.975$ | $4.80^{b} \pm 0.177$ |
| **FIR** | $1.00^{a} \pm 0.022$ | $0.51^{c} \pm 0.006$ | $0.72^{b} \pm 0.003$ |
| **CE** | $0.22^{a} \pm 0.000$ | $0.08^{c} \pm 0.020$ | $0.21^{b} \pm 0.007$ |
| **PER** | $0.54^{a} \pm 0.003$ | $0.21^{c} \pm 0.045$ | $0.52^{b} \pm 0.020$ |
| **PIR** | $0.40^{a} \pm 0.010$ | $0.20^{c} \pm 0.003$ | $0.29^{b} \pm 0.003$ |

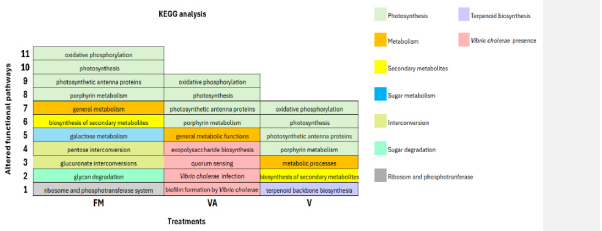


Figure 1. Number and types of altered functional pathways identified in the KEGG analysis after subjecting the fish to each of the experimental treatments.

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## Results and discussion

Growth performance and nutritional parameters of Chelon labrosus with experimental protein sources.

Individuals that were submitted to the V treatment showed an overall higher growth performance as well as more efficient nutritional rates, whereas the VA group provided the lowest zootechnical parameters values (Table 1). Furthermore, the alpha and beta diversity metrics confirmed the V diet had a significant impact on the fish microbiome, revealing distinct taxonomic differences compared to the other experimental groups.

As for the taxonomical groups found in the fish GIT, all experimental treatments modified the initial fish gut microbial community. However, slight biodiversity differences were observed between the three analyzed treatments (figure 1). Despite not having found any significant difference between the treatment's taxonomical biodiversity, the COGs and KEGGs terms analysis suggest that the experimental diets could have an effect in the fish gut microbial's community functionalities.

Although many previous works have studied the viability of providing innovative feeds with alternative protein sources to different intensive aquaculture species, little is known when *C.labrosus* becomes the target species of the nutritional study. Therefore, despite the lack of knowledge regarding this species' nutritional requirements, *C.labrosus* individuals reach optimal growth and zootechnical indexes when exclusively fed with vegetal protein-based diets. Such results could be due to the fact that inclusion of animal protein sources and hemoglobin affected the feed acceptability and palatability of the VA and FM pellets. This higher growth is aligned with a differential intestinal microbiota profile, as the *Pseudomonas, Methanobacter* and elusimicrobiaceae species that were exclusively found in the GIT from the V fish are known to have beneficial effects on nutrient digestion, fatty acids synthesis and immune defense boosting. In contrast*, Vibrio cholerae* proliferated in the fish gut of the VA specimens, which presence altered some physiological pathways (as observed in the KEGG analysis) and caused detrimental effects, having negative consequences to the fish growth and health.

## Conclusion

In summary, the manufacturing of plant-based diets for low trophic species like *C. labrosus* leads the aquaculture industry towards an optimistic future in which the sector reduces its dependency on fishmeal and fish oil, becoming truly sustainable.

## Acknowledgements

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# FERMENTATION OF INDUSTRIAL BY-PRODUCTS FOR HIGH-NUTRITIONAL-VALUE PRODUCT OBTENTION

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## Introduction

Spontaneous fermentation of different food industry by-products has been proved to yield a product with enhanced nutritional value. What is more, this process is known to increase some substrate's protein and enrich their amino acids profile. Furthermore, it has also been confirmed that fermentation of cereals and legumes bioconverts their carbohydrates, making them more digestible (Adebo et al., 2022). Therefore, due to the current pursuit of a truly sustainable substitute for fishmeal in aquaculture emphasizes the need to find potential raw materials with high-nutritional value for aquafeed production.

## Materials and methods

Different by-products from animal and vegetal origin were prepared for spontaneous fermentation and kept at 22ºC for 7 days. Samples of each fermented material were taken after 1 and 7 days of fermentation in 5 ml aliquots and preserved frozen for further chemical analysis. Then, a proximal nutritional composition analysis was performed on every sample to determine their crude protein level. On the other hand, a metagenomic analysis was performed by extracting bacterial DNA from the fermented samples. Last, a high-throughput sequencing of the 16S rRNA gene was conducted with an Ilumina MiSeq platform in order to analyze the bacterial diversity by using Claudio Braulio shini program.

## Results and discussion

Most vegetal materials and yeasts increased their CP level after the spontaneous fermentation, whereas biofloc, all animal by-products and some cereals weren't enhanced by the process (Table 1). Alpha diversity metrics showed a gradual decline of the microbial biodiversity during the 7 days of the fermentation. Furthermore, shifts in diversity were observed between the fermented and unfermented bacterial communities, underscoring the impact of fermentation. Beta diversity metrics also showed significant differences in the fermented microbial communities and confirmed a restructuring of the microbial profile from the fermented and unfermented samples over time (Figure 1). The taxonomic composition analysis, dynamic changes at phylum and genera level were observed throughout the fermentation process.

Although fermentation of vegetal materials has been previously proved to enhance their nutritional value by making them more protein-rich, some of the tested cereals (rice, wheat gluten, corn and pea meal) experienced a decrease in their CP levels probably due to bacterial difficulties when digesting complex carbohydrate molecules. As for animal by-products, all tested substances rotted and suffered a decrease on their CP after just 24 hours of fermentation, maybe because bacteria cannot feed on matrixes with scarce carbohydrates. On the other hand, fermentation did cause an exponential rise of most plants and yeasts protein content. However, some vegetal materials performed better than others by reaching higher protein levels after 7 days of fermentation (soybean meal,flax), while the increase of bacterial protein in other substances stabilized or stopped after 24 hours of fermentation (cameline, beetroot). For this reason, further research needs to be done to optimize spontaneous fermentation, as many authors claim that the addition of exogenous bacteria may boost the process and enrich the resultant products with

*(Continued on next page)*

Table 1: Crude protein expressed as dry matter percentage (CP (dm%)) of the different tested materials before and after the fermentation process.

| Raw materials | CP (%dm) BEFORE fermentation | CP (%dm) AFTER fermentation | | CP gain (%) |
|---|---|---|---|---|
| | | 1 day | 7 days | |
| Rice | 10 | 5.2 | 7.5 | None |
| **Degreased cameline** | 38.2 | 40 | 40.5 | 6.02 |
| **Degreased canola** | 39.9 | 41.4 | 42.8 | 7.39 |
| **Sunflower** | 20.6 | 20.8 | 21.3 | 3.4 |
| **Beer yeast 40%** | 45.2 | 45.5 | 46.6 | 3.1 |
| **Beer yeast 100%** | 48.3 | 48.2 | 48.7 | 0.83 |
| **Flax** | 21.5 | 21.9 | 24 | 11.7 |
| **Beetroot** | 8.7 | 9.7 | 9.8 | 13.64 |
| **Soybean meal** | 50.4 | 52.7 | 53.8 | 6.75 |
| ***Ulva lacinnlata*** | 20.6 | 20.2 | 21.7 | 5.35 |
| Wheat gluten | 93.3 | 85.7 | Dismissed | None |
| Peameal | 29.4 | 28.7 | Dismissed | None |
| Corn | 9 | No data | 8.2 | None |
| Wheat | 18.4 | 9.9 | Dismissed | None |
| Biofloc | 22 | No data | 20.6 | None |
| Shrimp heads | 74.8 | 69 | Dismissed | None |
| Shrimp bodies | 71.2 | 67.4 | Dismissed | None |
| Meat meal | 59.8 | 47.1 | Dismissed | None |
| Fish guts | 41.4 | 44.8 | Dismissed | 8.25 |
| Rainbow trout guts | 57.4 | 54.2 | Dismissed | None |

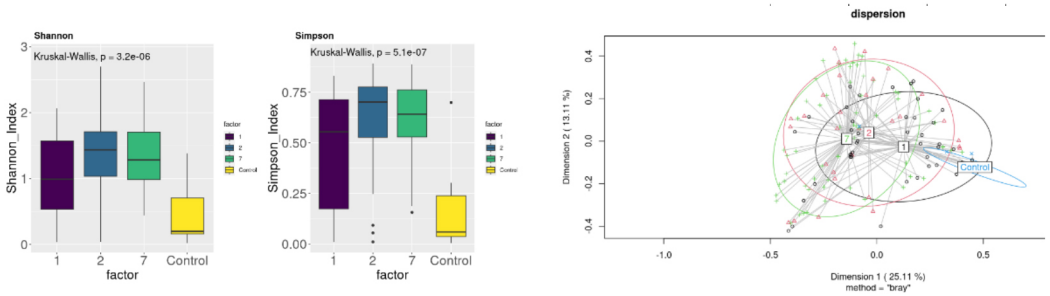


Figure 1. Alpha (left) and beta (right) diversity results of fermented materials at days 1, 2 and 7.

probiotics (Rajesh et al., 2010). As for the intestinal microflora population shifts, it is possible that the blooming of some taxons can also trigger the decline of others due to unsustainable competence for the same resources. Consequently, additional inquiry is required to determine how different microbial species may affect each other.

## Conclusion

Vegetal materials are more efficient at fermenting than other animal and biofloc by-products, yielding a product with enhanced protein content that can be used as a potential ingredient for aquafeeds. Nevertheless, further research needs to be done to optimize the fermentation process and test its potential to enhance alternative aquafeed ingredients.

## Acknowledgments

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# RECYCLING OF BIOFLOC AS NATURAL FOOD TO PRODUCE *Tenebrio molitor*

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## Introduction

Biofloc can be used as a source of live feed for farmed organisms. Furthermore, its excess and by-products have been also used successfully as a feeding source for insects, such as *Hermetia illucens* (Zhang et al., 2023). What is more, feeding larvae with biofloc waste may be a sustainable strategy which does not only help to recycle aquaculture's waste but that also converts it into a valuable alternative protein source (APS). Such approach would help lead aquaculture towards more sustainable practices that also align with the circular economy principles. Consequently, there is a current need to assess biofloc by-products as a potential feed for larvae to confirm if those insects can be turned into a nutritious protein-rich flour for aquafeed production.

## Materials and methods

*Tenebrio molitor* larvae were farmed in plastic containers for two weeks in a room with controlled environmental parameters. All experimental groups were fed wheat bran as dry feed. The control group received carrots twice a week as a source of hydration, while the other experimental groups were given biofloc 1, 2, or 3 times per week as substitute for the carrot. Larvae weight, non-eaten food weight and feces weight were measured weekly. At the end of the experimental trial, zootechnical parameters such as weight gain, mortality rate, feed conversion ratio (FCR) and frass production ratio (FPR) were calculated. Larvae were frozen and stored for their posterior nutritional composition analysis.

## Results and discussion

As shown in Table 1, control groups reached higher final biomass levels and provided lower FCR and FPR values during the first and second week of the experimental trial, reaching bigger larvae sizes too (Figure 1). In contrast, biofloc and wheat bran fed groups showed higher FCR and FPR values, indicating a lower feed conversion efficiency and confirming that some of the administered biofloc was not turned into biomass. However, biofloc was consumed at a higher rate when administered more frequently and no mortality rate could be observed in any of the experimental groups (Figure 2).

Although no literature has been found regarding biofloc's potential as a moisture source for insect feeding, one study evidenced that biofloc by-products mixed with wheat bran provided optimal growth results when tested as an alternative dry diet for blacksoldier fly larvae (Zhang et al., 2023). Despite the fact that fly larvae performed better than *T.molitor* when fed with the above-mentioned mix, our carrot-fed group provided similar results to other works in which mealworms were fed different moist raw materials (such as tangerine shells or mushroom spent substrate) (Kim et al., 2017). Therefore, even though biofloc may be a nutrient-rich substrate with probiotic effects, mealworm larvae seem to prefer, digest and assimilate better other moist ingredients like carrots. Nevertheless, biofloc may still be a promising dry ingredient when mixed with vegetal by-products.

## Conclusion

Although biolfoc by-products can be used as a suitable and safe ingredient for *T.molitor* larvae feeding, carrots were a more effective moisture source. Nevertheless, biofloc may still be a potential nutrient-rich ingredient for larvae feeding when mixed with other dry raw materials.

*(Continued on next page)*

Table 1: Zootechnical parameters results expressed as mean ± standard error (n=3). Different letters in each row indicate significant differences at p<0.05.

| | 7 days | | | | 14 days | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Biofloc 1 | Biofloc 2 | Biofloc 3 | Control | Biofloc 1 | Biofloc 2 | Biofloc 3 |
| Initial biomass (g) | 49.60 ± 0.001 | 49.60 ± 0.001 | 49.60 ± 0.001 | 49.60 ± 0.001 | 49.60 ± 0.001 | 49.60 ± 0.001 | 49.60 ± 0.001 | 49.60 ± 0.001 |
| Final biomass (g) | $90.78^{a}$ ±0.647 | $71.29^{c}$ ± 0.615 | $78.23^{b}$ ±0.133 | $79.10^{c}$ ± 0.182 | $67.86^{b}$ ± 0.301 | $71.05^{a}$ ±0.089 | $70.92^{a}$ ±0.193 | $71.33^{a}$ ±0.647 |
| Larvae weight gain (g/unit) | $0.056^{a}$ ± 0.001 | $0.032^{c}$ ± 0.001 | $0.039^{b}$ ± 0.001 | $0.038^{b}$ ± 0.001 | $0.046^{a}$ ± 0.301 | $0.040^{b}$ ±0.002 | $0.038^{c}$ ±0.002 | $0.047^{a}$ ±0.002 |
| FCR | $2.51^{a}$ ± 0.498 | $3.78^{c}$ ± 0.063 | $2.93^{b}$ ± 0.006 | $2.82^{b}$ ± 0.001 | 3.24 ± 0.163 | 3.55 ±0.050 | 3.42 ±0.048 | 3.36 ±0.301 |
| FPR | $1.59^{a}$ ± 0.016 | $2.72^{c}$ ± 0.047 | $2.35^{b}$ ± 0.012 | $2.37^{b}$ ± 0.001 | $2.74^{a}$ ± 0.029 | $2.88^{b}$ ±0.042 | $3.02^{b}$ ±0.009 | $3.12^{c}$ ±0.301 |
| Survival (%) | $96^{b}$ ± 0.1 | $96^{b}$ ± 0.1 | $97^{a}$ ± 0.1 | $98^{a}$ ± 0.1 | $98^{b}$ ± 0.1 | $99^{b}$ ± 0.1 | $100^{a}$ ± 0.1 | $97^{c}$ ± 0.1 |

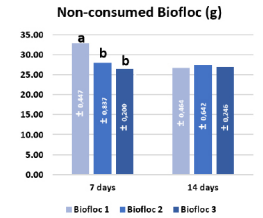


Figure 2: Non-consumed Biofloc (g) by experimental groups at 7 and 14 days. The letter above each column indicates significant differences at p<0.05. Tukey

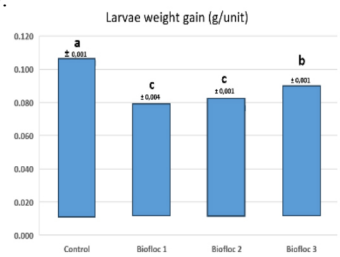


Figure 1: Overall larvae weight gain at the end of the experimental trial. Parameters are expressed as mean ± standard error (n=3) The letter above each column indicates significant differences at p<0.05. Tukey HSD test.

## Acknowledgments

This work was supported by I+D+i Research Project: "Pursuing the zero waste in Biofloc Technology to produce a sustainable and biosafe aquaculture product (ZeroFloc)" (PID2023-149570OB-I00). J. Gómez-Aguilera contract was supported by European Union Next Generation-Plan of Conselleria d'innovació, Universitats, Ciència i Societat Digital of Generalitat Valenciana (INVEST/2022/434).

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# PREDICTIVE MODELING OF FISH HEALTH IN AQUACULTURE USING CLINICAL CHEMISTRY BIOMARKERS

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**Introduction**
Implementing an automated biomarker-based approach with machine learning model enables real-time assessment, early detection of health issues and timely interventions to reduce mortality and improve economic outcomes. This study aimed to develop automated machine learning models capable of predicting overall fish health as well as specific health challenges, including gill health, viral health and bacterial infections.

**Methods**
Blood biomarker data were collected biweekly from multiple pens across different fish farms by WellFish Tech laboratories, encompassing 8,846 individual fish samples. The data was annotated for use in models targeting general fish health, gill health, viral health, and bacterial infections.

The dataset underwent data curation to ensure quality and consistency. Outliers were filtered and exploration data analysis using box plots was conducted to assess the distribution of each biomarker. Further, selection of features for the modeling was conducted with correlation matrices to retain only one feature of identified highly correlated biomarkers. Standard scaling was applied to all features. To address class imbalance, multiple techniques were employed to generate 4 different modified datasets including no rebalancing, under sampling, oversampling and SMOTE. The dataset was split 80/20 for training and testing. Following this, both Logistic regression and random forest classification models were evaluated on the four datasets. Model performance was assessed using accuracy, precision, recall, and F1-score.

**Results**: Tables below summarize the results of the performance metrics for each health model:

**Table 1. Fish Health Classification**

| Model | Rebalancing | Accuracy | Precision | Recall | F1 Score |
|---|---|---|---|---|---|
| Logistic Regression | No Rebalancing | 0.85 | 0.87 | 0.97 | 0.91 |
| Logistic Regression | Oversampling | 0.78 | 0.80 | 0.75 | 0.77 |
| Logistic Regression | Under sampling | 0.81 | 0.83 | 0.76 | 0.79 |
| Logistic Regression | SMOTE | 0.80 | 0.83 | 0.76 | 0.79 |
| Random Forest | No Rebalancing | 0.94 | 0.94 | 0.98 | 0.96 |
| Random Forest | Oversampling | 0.97 | 1.00 | 0.95 | 0.97 |
| Random Forest | Under sampling | 0.90 | 0.89 | 0.90 | 0.90 |
| Random Forest | SMOTE | 0.95 | 0.96 | 0.94 | 0.95 |

**Table 2. Gill Health Classification**

| Model | Rebalancing | Accuracy | Precision | Recall | F1 Score |
|---|---|---|---|---|---|
| Logistic Regression | No Rebalancing | 0.82 | 0.84 | 0.79 | 0.81 |
| Logistic Regression | Oversampling | 0.85 | 0.86 | 0.82 | 0.84 |
| Logistic Regression | Under sampling | 0.83 | 0.84 | 0.80 | 0.82 |
| Logistic Regression | SMOTE | 0.84 | 0.85 | 0.81 | 0.83 |
| Random Forest | No Rebalancing | 0.92 | 0.93 | 0.91 | 0.92 |
| Random Forest | Oversampling | 0.96 | 0.97 | 0.95 | 0.96 |
| Random Forest | Under sampling | 0.90 | 0.91 | 0.88 | 0.89 |
| Random Forest | SMOTE | 0.94 | 0.95 | 0.93 | 0.94 |

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**Table 3. Viral Health Classification**

| Model | Rebalancing | Accuracy | Precision | Recall | F1 Score |
|---|---|---|---|---|---|
| Logistic Regression | No Rebalancing | 0.75 | 0.77 | 0.70 | 0.73 |
| Logistic Regression | Oversampling | 0.78 | 0.80 | 0.74 | 0.77 |
| Logistic Regression | Under sampling | 0.76 | 0.78 | 0.72 | 0.75 |
| Logistic Regression | SMOTE | 0.79 | 0.81 | 0.75 | 0.78 |
| Random Forest | No Rebalancing | 0.92 | 0.93 | 0.90 | 0.91 |
| Random Forest | Oversampling | 0.95 | 0.96 | 0.94 | 0.95 |
| Random Forest | Under sampling | 0.91 | 0.92 | 0.89 | 0.90 |
| Random Forest | SMOTE | 0.93 | 0.94 | 0.92 | 0.93 |

**Table 4. Bacterial Infection Health Classification**

| Model | Rebalancing | Accuracy | Precision | Recall | F1 Score |
|---|---|---|---|---|---|
| Logistic Regression | No Rebalancing | 0.78 | 0.80 | 0.74 | 0.77 |
| Logistic Regression | Oversampling | 0.81 | 0.83 | 0.77 | 0.80 |
| Logistic Regression | Under sampling | 0.80 | 0.82 | 0.77 | 0.79 |
| Logistic Regression | SMOTE | 0.82 | 0.84 | 0.79 | 0.81 |
| Random Forest | No Rebalancing | 0.91 | 0.92 | 0.89 | 0.91 |
| Random Forest | Oversampling | 0.94 | 0.95 | 0.93 | 0.94 |
| Random Forest | Under sampling | 0.90 | 0.91 | 0.88 | 0.89 |
| Random Forest | SMOTE | 0.93 | 0.94 | 0.92 | 0.93 |

**Conclusion**

Automated machine learning models using blood biomarker data effectively predict fish health and specific disease challenges. Summary results in Table 1 to 4, Random Forest models with oversampling demonstrated superior performance across all health categories. This approach facilitates early detection and intervention, reducing mortality and enhancing operational efficiency in aquaculture.

# FIRST STEPS ON *Saccorhiza polyschides* CULTIVATION IN PORTUGAL´S SOUTHERN COAST

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## Introduction

The pseudo kelp *Saccorhiza polyschides*, commonly known as furbelow, is a fast-growing, annual brown seaweed with potential applications in agriculture and human nutrition. In addition to its reported uses as a fertilizer and biostimulant (Nhhala *et al.*, 2024), it is also considered an edible species in Northern Portugal, Galicia, and other parts of the world (Pereira, 2016). Recent studies have shown that both wild and cultivated *S. polyschides* contain significant levels of essential nutrients, exhibit anti-inflammatory activity, and are rich in key mineral elements (Cardoso et al., 2023). Morphologically, *S. polyschides* is characterized by a large blade growing to over 2m long, with a flattened stipe with wavy frills, and a distinctive hollow bulbous holdfast. Its life cycle resembles that of the Laminarians, alternating between macroscopic diploid sporophytes and microscopic haploid gametophytes (FAO, 1970).

Within Vertical Algae project protocols for *S. polyschides* cultivation are being developed and optimised, in both nursery and at sea, in association with a seabream and seabass fish farm in the Southern coast of Portugal (Seaculture Lda. IMTA DEMO), exploring the potential of integrated multi-trophic aquaculture (IMTA) systems.

## Methods

Mature sori of *S. polyschides* were collected along the Portuguese coast between July and December of 2023 and 2024. Spore release and cultivation techniques were adapted from established protocols for kelp species (e.g., Edwards *et al.*, 2011). Twine seeding followed the direct seeding method from spores and seeded twine was deployed at Seaculture Lda. IMTA DEMO site.

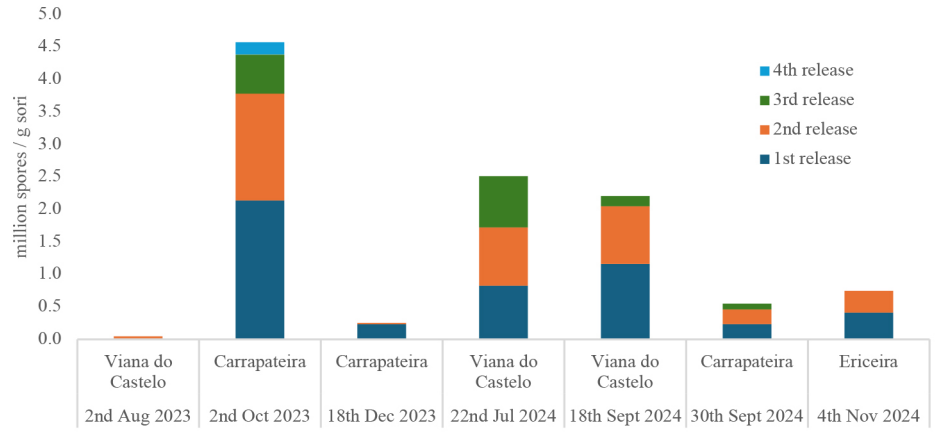


Figure 1 - Summary of *S. polyschides* spores release events during 2023 and 2024.

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**Results & Discussion**

Spore release for the several sori collection are presented in Figure 1, with the observed values of spores/g sori at a single re-hydration event varying between 17,316 and 2,137,394 spores/g sori.

Twine in spools was seeded with spores in October 2023, September, October and November 2024. Due to electrical failure and contamination at the nursery, spools seeded in September and October 2024 did not develop into healthy sporophytes and were discarded. In October 2023, the seeded twine was cultivated at 3 different temperatures (14, 16 and 18ºC), a closer monitoring at nursery was performed and it was observed the effect of temperature in the dramatic increase in contamination, with spools reared at 14ºC displayed the best resilience and lowest amount of contamination, being this the temperature chosen for all following cultivation. Also, sporophytes reached the minimum deployment size (~2-3mm) 29 days post-inoculation and remained healthy for 64 days. The twine seeded in October 2023 was deployed at sea mid-December 2023, and twine seeded in November 2024 was deployed at sea mid-February 2025.

While *S. polyschides* individual growth achieved at sea was promising, given the cultivation conditions and deployment, the density observed at harvest was below expectations, indicating the method needs further optimization. To address the challenges encountered, namely contamination in the nursery and low densities at sea, future work will focus on gametophyte cultivation and gametophyte/sporophyte seeding.

**Acknowledgments**

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# *Ulva sp.* CULTIVATION IN LAND-BASED IMTA

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BLUEBOOST aims to demonstrate that co-culturing a wide range of low trophic species with established species can enhance European aquaculture production of blue foods and feeds, while reducing the environmental footprint and moving towards a carbon-neutral blue economy in aquaculture.

One of the six IMTA demonstration systems within BLUEBOOST is the cultivation of *Ulva* sp. integrated into a land-based pilot fish production system in the Algarve, South Portugal. In IMTA, species from different trophic levels are cultivated in proximity so that the waste from one species is recycled and becomes an input for another (Chopin *et al.*, 2001). This way, extractive species, such as seaweeds (macroalgae), can remove dissolved inorganic nutrients waste from fed culture and reduce its ecological impacts while providing the farmer with a new product. In parallel, seaweed biomass produced in IMTA has the potential to contribute directly to food security, as sustainable food, feed ingredients, or agricultural biofertilizers/biostimulants (e.g. Cai *et al.,* 2021; Craigie, 2011, Magnusson *et al.*, 2019). The genus Ulva (Ulvales, Ulvophyceae, Chlorophyta) shows a cosmopolitan distribution, including Portugal, and the Algarve , characterized by high growth rates and with a wide commercial interest. Several studies have focused on Ulva cultivation in IMTA and demonstrated its high, but very variable, potential for biomass production and nitrogen removal, besides most available data refers only to small-scale experimental production. Within this project the scale up of Ulva cultivation in earthen ponds in the South of Portugal will be tested and its productivity and bioremediation potential evaluated.

Initial biomass of *Ulva* sp. was harvested from EPPO-IPMA facilities (South Portugal) and transferred to a ~600m$^2$ earthen pond with aeration and receiving effluent from the research station fish production. Samples of the collected *Ulva* sp. were taken for molecular species identification, reproductive status, FW:DW ratio and C, N and P (CNP) composition. In the cultivation pond, temperature, pH, salinity and light measurements are taken every 10 mins via HOBO loggers, while turbidity and dissolved oxygen are measured daily (5 days per week). Water samples are collected twice a week at the inflow and outflow for dissolved nutrients analysis. Every 4 weeks *Ulva* sp. yield is assessed and excess biomass removed, with subsamples analyzed for reproductive status, FW:DW ratio and CNP content. With yield and CNP data, the bioremediation potential of *Ulva* sp. for nutrient removal will be quantified over the production period.

Overall, this study will provide insights into the feasibility of scaling up Ulva cultivation in IMTA systems, demonstrating its dual role as a biomass resource and a tool for nutrient bioremediation in land-based aquaculture

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**Acknowledgments**
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# ISOLATION AND GENOME ANALYSIS OF THE NOVEL BACILLUS BACTERIOPHAGE JAYAN

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Introduction:
Commercial aquaculture probiotics commonly use *Bacillus licheniformis*, a probiotic strain, to boost beneficial microbial loads in prawn farms, thereby boosting productivity and providing safe food for consumers (Simmonds *et al.*, 2023). If commercial probiotics in aquaculture farms become infected by their specific phages, their population could eventually diminish instead of expanding. We are currently not sure to what degree phages threaten the effectiveness of probiotic bacteria. Phage isolation against probiotics is crucial for both understanding the viral ecology of probiotics and generating probiotic strains that are resistant to bacteriophage infection. Here we present a novel phage discovered against *Bacillus licheniformis*, Jayan.

Materials and Methods:
Aquaculture probiotic strains were isolated from three Bangladeshi commercial probiotic products using spread and streak plate methods. Genomic DNA extracted with the Beat-Beat Micro AXE kit and was sequenced on the Oxford Nanopore PromethION96 platform. Reads were trimmed (Porechop), assembled (Flye), annotated (PROKKA), and analysed using BV-BRC, TYGS, and visualized using iTOL. Phages were enriched from Danish wastewater and organic waste using a modified enrichment method (Van Twest and Kropinski, 2009), purified by repeated double agar overlay, amplified, and extracted DNA prepared with the NEBNext Ultra II FS kit for Illumina iSeq. Reads were processed with CLC Genomic Workbench, annotated with Pharokka, compared using VRIDIC and Clinker, then phylogenetically assessed with ViPTree. The spot test method was employed to evaluate the host range of phage Jayan against isolated commercial aquaculture probiotic strains.

Results and Discussion:
According to the TYGS phylogenetic analysis of these genomes, we identified ten morphologically distinct colonies belonging to the *Bacillus* and *Lysinibacillus* genus. These colonies encompass six distinct species, namely *Bacillus subtilis*, *Bacillus paranthracis*, *Bacillus paralicheniformis*, *Bacillus safensis*, *Bacillus licheniformis*, and *Lysinibacillus telephonicus*. For further confirmation of these results, a comprehensive genome analysis was conducted employing BV-BRC comprehensive genome analysis tools. The Jayan host strain *B. licheniformis* contains 4578 protein coding sequences (CDS), 80 transfer RNA (tRNA) genes, and 24 ribosomal RNA (rRNA) genes. This probiotic strain exhibited 97% query coverage and 99.93% identity with a 0.0 E value to the *B. licheniformis* DSM13 (NC_006322.1) strain during NCBI blastn with align two or more sequences option between isolated probiotic strain and GenBank *B. licheniformis* DSM13 strain.

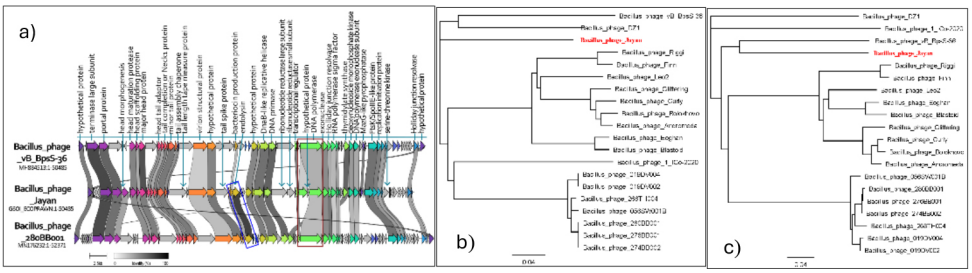


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Figure 1. a) The Clinker map demonstrates the genomic synteny between the isolated phage and two highly closest relatives. The genome sizes, measured in base pairs (bp), of each phage are listed below. The maroon color rectangle indicates DNA polymerases of Jayan with closely related Bacillus phages, and dark yellow arrows in blue color rectangle denotes the endolysin; b) The phylogenetic tree of phage Jayan derived from terminase large subunit, and c) The phylogenetic tree of phage Jayan based on DNA polymerase.

Phage Jayan (PQ299153.1), isolated from organic waste, forms asymmetrical plaques on *B. licheniformis*, has a short latent period (10–15 min) and 25 min burst, and inhibits *B. licheniformis* DSM13 but not most other Bacillus spp. Genomically, Jayan (47,420 bp; 43.28% GC) shares only 20–27% intergenomic similarity with known phages , placing it as a novel species in a new *Herelleviridae* genus. Its DNA polymerase is disrupted by a unique 270 bp insertion (Figure 1a), while it retains an endolysin related to Bacillus phage 280BB001 (Loney *et al.*, 2023); phylogenetic analyses (Figure 1b & 1c) link conserved genes (TLS, primase) to phages DZ1 and vB_BpsS-36 (Akhwale *et al.*, 2019), but overall divergence confirms Jayan as a distinct lineage.

Conclusion:

Jayan is a novel virulent phage of *B. licheniformis* within a new *Herelleviridae* genus, isolated from organic waste in Copenhagen, with unique genetics and implications for probiotic ecology and phage-resistant strain development.

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# INTEGRATION OF BLACK SOLDIER FLY LARVAL MEAL IN THE DIET OF *Cyprinus carpio*: GROWTH, ENZYMATIC ACTIVITY, AND INTESTINAL HEALTH ASSESSMENT

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**Introduction**

Aquatic protein production plays a vital role in meeting the growing global demand for protein, which ensures food and nutrition security (Melenchón et al., 2022). According to FAO, more than 70% of aquaculture production currently depends on fishmeal (FM)-based feeds. As aquaculture expands rapidly, finding sustainable alternatives to traditional FM is becoming increasingly crucial (Fabrikov et al., 2021). The findings of Broeckx et al., (2021) and in line with the UNSDGs, insect meal (IM) has been emerged as promising and environmentally friendly substitute for aquafeeds (Quang et al., 2022). Studies reported that the novelty foods in many developed countries are insect-based and are now gaining attraction, particularly in Asia. This shift is supported by initiatives such as the establishment of the Asian Food and Feed Insect Association (AFFIA) in Bangkok, which promotes IM production across the region. Thus, IM has emerged as a promising and environmentally sustainable replacement for FM in aquafeeds (Quang et al., 2022), offering a renewable source of protein for food, feed, and biomaterials. In this context, sustainable and circular solutions required to ensure the long-term viability of aquafeeds. This study aimed to explore the potential of *Hermetia illucens* - black soldier fly larvae (BSFL) as an alternative protein source in fish diets through an integrated biowaste management approach using onion waste. The findings of the study focused on three primary objectives: (i) evaluating the feasibility of utilizing BSFL to upcycle onion waste, (ii) assessing the nutritional suitability of BSFL meal in fish feed, and (iii) comparing the environmental implications of BSFL meal inclusion versus conventional fishmeal.

**Materials and Methods**

In this study, BSFL were reared on a substrate composed of onion waste and poultry manure under controlled laboratory conditions. The bioconversion efficiency, larval growth, and compost quality were assessed through proximate analysis. Five isonitrogenous diets replacing fishmeal with BSFL meal at 0%-D0, 25%-D1, 50%-D2, 75%-D3, and 100%-D4 levels were formulated and analyzed for nutritional and physical properties, including sinking velocity and water stability. A feeding trial was conducted using *Cyprinus carpio* fingerlings for 30 days, during which growth performance, feed utilization, and survival were monitored. Blood samples were analyzed for hematological and biochemical parameters, while liver and intestinal tissues were examined for digestive enzymes (amylase, protease, trypsin) and antioxidant enzymes (SOD, CAT, GSH, GPx) activities. Histological analysis of the distal intestine was performed to observe structural changes due to BSFL inclusion. All tests were conducted in three biological triplicates. Data presented as mean ± standard deviation (SD). Data were compared using one-way analysis of variance (ANOVA) and post-hoc comparison test (DMRT) using GraphPad Prism version 9 (GraphPad Software, Inc., San Diego, CA, USA).

**Results**

BSFL were reared on a mixed substrate of onion waste and poultry manure under controlled laboratory conditions. Larval growth, bioconversion efficiency, survival rates, and compost quality were systematically evaluated. The rearing process demonstrated high larval survival (above 90%) and significant substrate degradation, highlighting the effectiveness of BSFL in waste valorization. Nutrient profiling confirmed that BSFL had high crude protein (38.47%) and lipid content (48.79%), making it a viable feed ingredient. Subsequently, five isonitrogenous diets (D0 to D4) were formulated by progressively replacing fishmeal with BSFL meal at 0%, 25%, 50%, 75%, and 100% levels, respectively. These diets were fed to *C. carpio* fingerlings over a 30-day period. Growth performance indicators such as weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and viscerosomatic index (VSI) were significantly improved in fish fed the 50% and 75% BSFL diets (D2 and D3), without compromising survival. Hematological and biochemical parameters remained within physiological ranges across all diets, suggesting that BSFL inclusion did not induce systemic stress. Digestive enzyme activities

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(amylase, protease, trypsin) and antioxidant enzyme levels (SOD, CAT, GSH, GPx) were also enhanced in BSFL-fed groups, particularly D2, indicating better digestion and oxidative stress management. Histopathological analysis of intestinal tissues revealed mild to moderate inflammation in higher inclusion groups (D3 and D4), warranting careful monitoring of gut health when formulating high-BSFL diets. Nevertheless, the overall findings validate the use of BSFL meal as a sustainable protein alternative, capable of replacing up to 50–75% of fishmeal without adverse effects on fish growth or health. Moreover, the frass (larval compost) generated post-bioremediation proved to be a nutrient-rich organic fertilizer, supporting soil sustainability.

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# A NEW PERSPECTIVE ON ELECTRICAL STUNNING AND LIVE-CHILLING IN THE INDUSTRIAL SLAUGHTER OF TURBOT: INSIGHTS FROM ELECTROENCEPHALOGRAPHY

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## Introduction

Current animal welfare guidelines, based on the five-domain framework, state the need to prevent animal suffering, particularly during slaughter. The guidelines recommend that fish must be rendered unconscious through appropriate stunning methods such as live chilling, electronarcosis, or percussive stunning (OIE, 2019), but the effectiveness of these methods varies by species and requires validation. Consequently, turbot farmers have become interested in alternative methods to conventional live chilling, leading to studies that assessed electrical and percussive stunning using behavioural indicators. However, these indicators were found to be unreliable for determining consciousness due to their poor correlation with neurological activity (Morzel et al., 2003). In this context, electroencephalography (EEG) has been recommended as a preferred method to evaluate the effectiveness of stunning techniques (Bowman et al., 2019). The present study aimed to provide new evidence on the effects of electrical and live chilling stunning methods on turbot, using EEG to assess their effectiveness in industry settings.

## Materials and methods

Commercial-sized turbot (*Scophthalmus maximus*) weighing 1–2.5 kg from Stolt Sea Farm S.A. and CETGA (A Coruña, Spain) were used to evaluate the efficacy of industrial electric stunning and live chilling methods. Individually, each fish was anaesthetised with MS-222 (80 mg/L) prior to cranial placement of two stainless steel EEG electrodes over the telencephalon and optic lobe, with a reference electrode in the dorsal muscle. After recovery of the fish from anaesthesia, basal EEG recordings and strobe light-induced visual evoked responses (VERs) were collected for 10 min. For electrical stunning, the electrodes were removed prior to placing the fish in an industrial stunner (50 V, 50 Hz), and repositioned upon exit from the stunner, with brain activity being recorded for the next 15 min. For live chilling, recordings were taken at 10-min intervals before and during exposure to ice slurry, as well as after transfer to normal temperature water. EEG data were filtered to remove artefacts and analysed using Spike2 software, with power spectrum analysis (band pass filter 0.5-32 Hz, 10 Hz resolution) used to calculate total power (Ptot) and αβ power (Pαβ), both normalised to baseline. VERs were assessed by averaging twenty 1-second windows during light transitions.

## Results

This study evaluated the effect of two industrial stunning methods, electrical stunning and live chilling, on EEG activity in commercial-sized turbot. Our results showed that electrical stunning slightly increased power parameters values, although this was not significant due to the high variability of the recordings, especially towards the end of the post-stunning period studied. As for VERs, no attenuation or disappearance was evident following electrical stunning; on the contrary, a twofold increase in VER amplitude was noted during the early post-stunning recording.

Regarding live chilling, a progressive reduction in EEG power parameters was observed after immersion of fish in ice slurry. Significant decreases were observed within two minutes in ice slurry, reaching levels approximately 60% lower than those prior to cooling. VERs also showed a gradual decrease starting after two minutes of immersion in the ice mixture, disappearing complete after five minutes. Upon returning the fish to water of normal temperature, a gradual recovery of power parameters was observed, reaching pre-cooling levels after six min. The VERs also reappeared and gradually recovered to pre-cooling amplitude levels within two minutes of exposure to normal water temperature.

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**Discussion**

This study analysed and compared the EEG effects of electrical stunning and live chilling to assess their effectiveness in rendering turbot unconscious during industrial slaughter. We evaluated the electric stunning using the optimised set-up determined by previous operational indicator tests. The results showed that this method strongly immobilizes the fish by muscular blockage for a few min, but it does not cause reduction in brain activity and/or loss of VERs. This indicates that fish may remain conscious after stunning and highlights the poor correlation between behavioural signs and EEG-based measures of unconsciousness (Bowman et al., 2019).

On the other hand, live chilling resulted in a progressive decrease in brain activity, reaching a reduction of more than 50% in power parameters. In addition, a progressive disappearance of VERs within five minutes of live chilling was observed. These EEG findings are consistent with standards used in rainbow trout studies, where the absence of VERs is considered a reliable indicator of unconsciousness (Bowman et al., 2019), suggesting that this method could induce loss of consciousness in turbot. However, despite these results, live chilling also does not induce unconsciousness immediately, so with the current protocol it cannot be claimed to be an optimal method for stunning according to welfare guidelines (OIE, 2019).

Overall, our results show that the current method of electrical stunning is ineffective in rendering turbot unconscious, while live chilling appears more effective, although with time constraints that need to be considered. This study also highlights the limitations of operational indicators and emphasises the importance of using EEG techniques when assessing slaughter in turbot.

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# EXPERIMENTAL INVESTIGATION OF BOAT NOISE EFFECTS ON CAPTIVE BLUEFIN TUNA (*Thunnus thynnus*) BEHAVIOR

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**Introduction**

This study presents a controlled experimental campaign to observe any possible momentary behavioral changes in adult captive bluefin tuna in a tank, exposed to boat noise playback for durations of fifteen minutes. The goal is to assess whether such stimuli elicit measurable alterations in swimming patterns or group dynamics, given the tuna have limited response options because of the confined space inside tank in contrast to the open sea.

**Materials and methods**

The experiment was conducted during four days in a circular tank (22 m diameter, 9–9.5 m depth) that housed 17 adult bluefin tuna fish ranging from 200 to 250 kg at the ICTS-ICAR, (www.icar.ieo.es) tuna fish facilities in Mazarrón, Spain. The fish have grown up in the same facilities over many years. The fish typically swam in group and in a clockwise direction, counter to the water current generated by two pumps. The sound used in the experiment was a playback of a recording of a passing by boat noise 21 second long, which was played repeatedly for fifteen minutes. During the daytime period, intervals of 15 minutes were interspersed with a test and rest signal. The boat playback sound was emitted by an underwater loudspeaker (Lubell VC2C sound source) with three different levels being played with a gap of around 2.5 hours between each emission level. The levels were chosen to ensure adequate sound pressure level inside the tank as compared to the ambient noise as well as not as high as to scare the tuna that may result in collision with the walls. Apart from the boat playback sound measurements, the ambient conditions inside the tank were also measured. The experiment was repeated for four days in a similar fashion. The sound field was recorded with 3 hydrophones (AQUARIAN AS-1) and an autonomous hydrophone NAUTA used as a reference. Two stereoscopic cameras were used to record the movements of tuna during the sound recordings. The proposed experimental setup is shown in figure 1.

The emitted levels were chosen to ensure adequate sound pressure level inside the tank as compared to the ambient noise. Figure 2 (left) shows the comparation between the SPL with hand without sound source. The cameras were installed on opposite sides of the tank at mid-depth to monitor fish behavior, ensuring synchronized visual and acoustic data collection and, on the right is showed the SPL in a period of fighting minutes when the test sample is emitting.

**Results and discussion**

An application has been developed through which the spectrogram, the temporal evolution of the SPL and a video of the tuna can be displayed at the same time for a set period (figure 3). The camera recording, though not covering the whole tank and hence fish not visible all the time, shows that the fish show slightly faster movement with the boat noise radiation as compared to the ambient conditions. The most obvious reactions in behavior occur in the transients of rising emissions where there is an increase in SPL of around 20 dB (depending on the position of the hydrophone).

**Conclusion**

The observed behavioral response of captive bluefin tuna to boat noise playback was subtle, characterized primarily by a slight increase in swimming speed rather than disorganized or erratic movement. This suggests a level of acoustic stimulation that does not cause a pronounced stress reaction, which may be influenced by the confined nature of the tank. However, clear reactions to some rising transients have been found, showing changes in the direction of turns and disorder in the group dynamics, which must be confirmed in future experiments with higher sound levels in the test signals.

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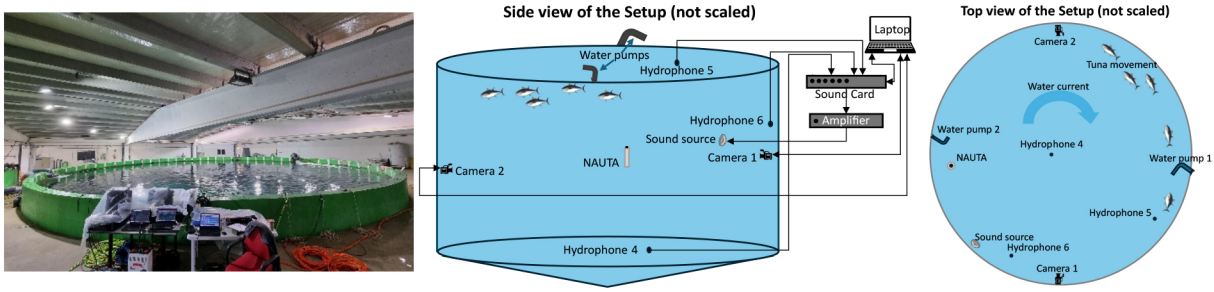


Figure 1. Experimental Setup. Left: Overview of the tank and the location of control equipment. Center: Side view. Location of emitter and receiver transducers. Right: Top view of the setup.

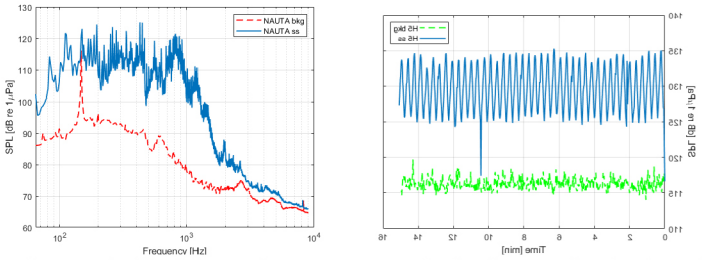


Figure 2. Sound pressure levels recorded by Nauta. As you can see, the background noise is well below that of the emitted signal.

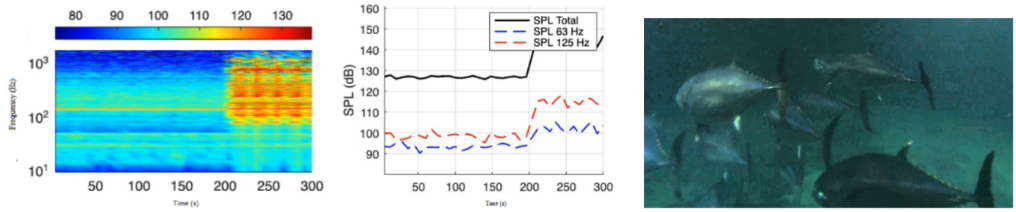


Figure 3. Information provided by the implemented application: Left: Spectrogram. Center: Temporal evolution of SPL. Right: Images of tuna.

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# ABOUT THE EXPERIMENTAL SETUP FOR THE EFFECTS OF SOUD ON CAPTIVE JUVENILE FISHES BEHAVIOR

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**Introduction**

This paper analyzes some key points in experimental configurations used to evaluate the behavior of juvenile vertebrate fish in tanks, taking as a reference an experience carried out in 2024 in which eight tanks were used, four control tanks and four that were subjected to an increasing level of sound exposure. Samples were taken at 30 minutes, 3 hours, 24 hours and 7 days. Biological issues are left aside for other work. This only affects some technical considerations/precautions to be considered.

**Materials and methods**

As mentioned above, eight tanks were used arranged as shown in Figure 1 (four control tanks and four experiment tanks).

Before starting the experiment, a series of operations have been carried out and some decisions have been made, which are discussed below. Some of these decisions/operations condition the others and have not been taken successively.

**Acoustic Analysis of the Tank.** A numerical model (FEM) with the same dimensions as the experimental tank. The tank was modeled using acoustic and shell (structure mechanics) module with a fluid-structure interaction boundary between the common boundaries. With this analysis, it was possible to obtain the response of the tank and compare it with experimental measurements.

**Selección de la señal de prueba**. In the experiment on fish six audio recordings of different approaching boats were played randomly. Four of the recordings were 2 minutes 45 seconds long, one was 2 minutes 25 seconds, and the last one was 3 minutes and 56 seconds long. All the audios are recordings of boats approaching and passing by.

**Selection/design of the sound source**. Figure 2 shows the parts of the DML (Distributed Mode Loudspeaker) radiant system designed. The figure on the left shows the actuator, the one on the center-right the container and the one on the center right the vibrating and radiating plate that is excited by the actuator. Figure 2 on the right shows the final aspect of the speaker. This speaker is designed to radiate from the surface of the water.

**Channel calibration and adjustment**. The calibration of the receiver transducers is described in standardized procedures. Often the channel setting in the broadcast part is not considered. In a cost-effective configuration such as the one described, the key point is in the connection between the board and the power amplifier since depending on the input sensitivity of the amplifier and the output level of the preamp, distortions of different types can occur.

**Conclusion**

The development of an experimental configuration for the study of the effect of noise on fish involves a series of operations among which it is not possible to avoid: a) the performance of prior numerical analyses to ensure that the contribution of the enclosure is known (acoustically speaking) and that the dynamic margin to carry out the experiment is known; and (b) the electroacoustic adjustment of the channel to ensure that the equipment is being used under conditions of minimum possible distortion.

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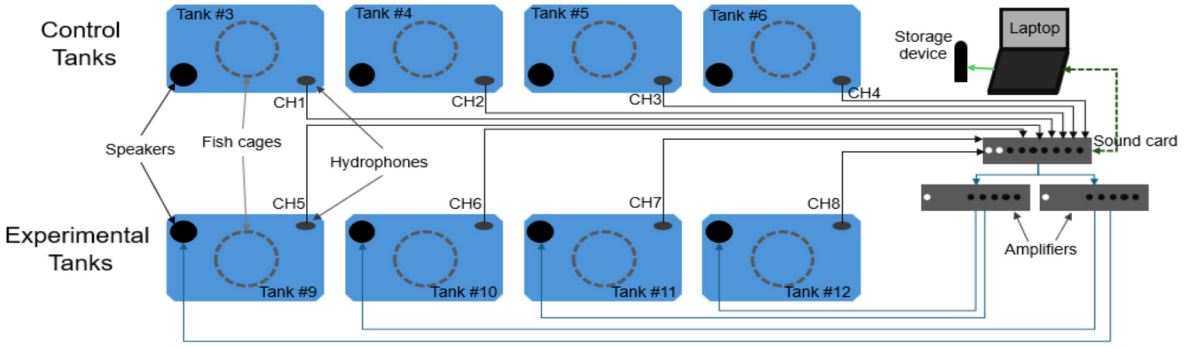


Figure 1. Experimental Setup consists of eight tanks, a sound card with eight inputs and outputs and the respective amplifiers and a computer for control.

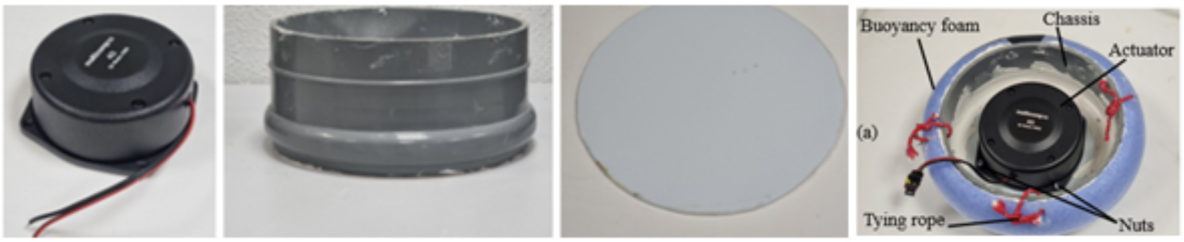


Figure 2. the DML (Distributed Mode Loudspeaker) radiant system designed for the experiment

Figure 3 ,left, show, the arrangement of the necessary elements in the tank (loudspeaker, net, where the fish are contained, and hydrophone) and, right, the SPLs of the four experimental tanks compared to the average level in the control tanks.

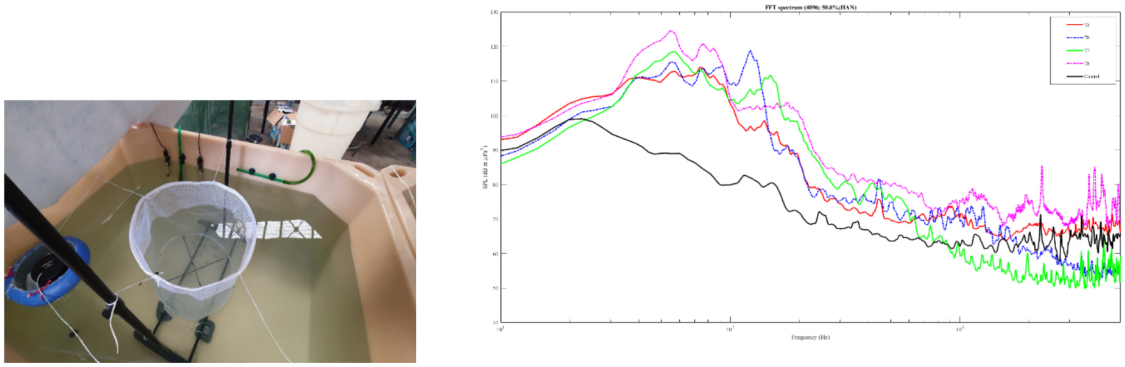

Figure 3. Left. Location of the different devices in the tank. Right. Sound pressure levels in each of the tanks compared to the sound pressure level in the control tanks

**Acknowledgments**

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# AQUASELF2. INTEGRAL ENERGY MANAGEMENT OF AQUACULTURE FACILITIES, GREEN HYDROGEN, ZERO DISCHARGE

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## Introduction

Currently, there is a high level of awareness regarding the use of renewable energy and its optimization in production processes. In aquaculture, there is a vast field of application, especially in the use of energy-sustainable RAS (Recirculating Aquaculture Systems). By determining the energy needs, a method for energy-sustainable operation can be established, allowing the culture of sea bass (*Dicentrarchus labrax*) to be carried out solely with renewable energy. Once the needs are identified, the necessary solar field and automation system have been designed, along with the volumes of tanks and treatment systems required for the cultivation. Another interesting factor is the incorporation of electricity generated by green hydrogen, which will be produced via photovoltaic methods. Additionally, due to the environmental issues caused by nitrogen waste (urine, feed residues), the goal has been set to prevent water discharge outside the system, significantly reducing the required energy input. To achieve this, biopolymers have been used in downflow reactors operating under semi-anaerobic conditions. Our results show that it is possible to cultivate sea bass during the ongrowing phase without water discharge and with 100% renewable energy.

## Materials and methods

The experiment was conducted at the facility of the Murcia Oceanographic Center (IEO, CSIC), in Puerto de Mazarrón (Murcia, Spain) (37º34'35" N 1º14'02" W). Five tanks of 1,680 liters were used, connected at their base to a biological treatment tank with a capacity of 1,850 liters. Temperature and oxygen concentration in the tanks were monitored daily, and $NH_4^+$, $NO_2^-$, $NO_3^-$ and pH were measured weekly. To eliminate nitrogenous compounds, a 1,850 liter tank (Denitrification Tank – "Tank-Des") was used for denitrification (Alzate Marín, 2019). In addition, one 1,680 liter tank was used as an electrochemical purification tank ("Tank-Pur"), where purification was carried out using solar electricity and a Ti-Rh electrode. In the Tank-Des, four downflow reactors were installed, each divided into two compartments. The lower compartment contained a support medium for denitrifying bacteria (porous pumice), while the upper compartment contained a biopolymer (starch-derived) synthesized in the laboratory.

A total of 125 European seabass (*Dicentrarchus labrax*) weighing 53 ± 20 g were distributed among five culture tanks, with 25 fish per tank. Seabass were fed manually to apparent satiety twice a day, at 09:00 and 13:00, with commercial feed D-3 Alterna 3p from Skretting (44% protein content) on alternate days. The following parameters were estimated: weight gain, specific growth rate (SGR = (ln (final weight / initial weight)) * 100 / days), feed conversion ratio (feed intake / weight gain), individual feed consumption, and the coefficient of variation in live weight at the beginning and end of the experiment (standard deviation / mean weight).

Two water recirculation systems were used. The first system supplied water directly to the five tanks after passing through a ring filter, cartridge filter, skimmer, ultraviolet sterilizer, blower (biological filter), and heat pump. The minimum target for daily water renewal was set at 200% of the water treated in the biological filter. Once the renewal flow rate and its treatment were established, a theoretical minimum energy consumption of 10 $kWh^{-1}$ per day was calculated for the most unfavorable periods (winter). The second recirculation system (denitrification) operated at a flow rate of 2 liters per minute, passing through a ring filter to provide anaerobic conditions in certain parts of the reactors. A 0.4 kW pump with a potentiometer regulator was used to set this flow rate, resulting in a theoretical consumption of 3 $kWh^{-1}$ per day under the most unfavorable conditions (winter). The culture tanks were cleaned by collecting coarse solids in the ring filter and returning the water to the denitrification tank. The retained particles were removed by cleaning with fresh water. Subsequently, the denitrified water was purified electrochemically using a Ti-Rh electrode and added back to the culture tanks after neutralization. Due to the lack of energy for cooling during summer, a reactor was designed for the generation of green hydrogen and oxygen, both separately. The oxygen was supplied to the culture tanks, and the hydrogen to a generator to produce electricity.

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**Results and discussion**

As established by Timmons *et al*. (2009), the emission rate of nitrogenous compounds is given by PNAT = F × %protein × %protein. In a fully closed-loop system, all nitrogen introduced must ultimately end up as $NO_3^-$ thanks to the biological filter. Dissolved protein in the water should be removed by the skimmer, while faeces and feed waste are eliminated through filtration and retention in the ring filter. The results are shown in the following table:

| | $NH_4^+$ ppm | $NO_2^-$ ppm | $NO_3^-$ ppm | Feed g | N supplied g | N present g | Nitrogen compound removal | Solar consumption $kWh^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| **January** | 0.5 | 0 | 0 | 250.0 | 30.0 | 4.1 | -25.9 | 185.9 |
| **February** | 0 | 0.5 | 15 | 810.5 | 97.3 | 127.9 | 30.6 | 438.8 |
| **March** | 0 | 0.5 | 20 | 1,074.8 | 129.0 | 169.1 | 40.2 | 419.0 |
| **April** | 0 | 0.5 | 35 | 1,941.9 | 233.0 | 292.9 | 59.9 | 459.0 |
| **May** | 0.35 | 0.8 | 25 | 2,975.6 | 357.1 | 215.7 | -141.3 | 398.0 |
| **June** | 0.35 | 0.8 | 30 | 3,252.0 | 390.2 | 257.0 | -133.3 | 741.0 |
| | | | | 10,304.8 | | | | |

The main forms of nitrogenous waste excretion include $NH_4^+$, as noted by Martínez López (2002). Currently, an adequate removal rate is expected to ensure proper operation of the culture system, with target values of $NO_3^- < 30$ ppm, $NO_2^- < 1$ ppm, and $NH_4^+ < 0.5$ ppm.

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**Acknowledgments**

To the staff of the Mazarrón aquaculture facility (IEO, CSIC). Without them, none of this would have been possible. This work has been funded by the THINKINAZUL project, co-funded by the Ministry of Science, Innovation and Universities NextGeneration EU funds (PRTRC17.I1) and by the Regional Community of Murcia - Fundación Seneca.

# IMPACT OF SHORTDURATION PRETREATMENTS ON DEWATERING EFFICIENCY OF BROWN SEAWEEDS

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Introduction

Seaweed biomass is an increasingly important sustainable resource for aquaculture feed. However, due to its *high-water content* (ranging between 74–89%) presents challenges by increasing transportation costs, spoilage, and reducing processing efficiency (Prahu et al., 2020). Mechanical dewatering complemented by pretreatment steps is therefore critical to improve dry matter content and biomass stability prior to downstream processing (Milledge and Harvey, 2016). Chemical pretreatments such as acid or alkali have been shown to alter seaweed tissue structure, affecting water binding and enhance biorefining process (Kadam et al., 2015). This work examines the short-term effects (< 5 min) of fresh water, alkaline (KOH), and acidic (HCl) soaking and mechanical pressing on seaweed dewatering performance, correcting for mass changes induced by pretreatment to provide an evaluation of pressing efficiency in moisture removal from fresh seaweed.

**Materials and Methods**

***Alaria esculenta*** *(AE)* and ***Saccharina latissima*** *(SL)* were farmed and harvested in June 2024 from Måsskjæra, Norway, and supplied by Seaweed Solutions used in this study. The fresh seaweed biomass was received as a block-frozen and transported to the processing facility. Prior to pretreatment, each seaweed sample was thawed to room temperature. Four pretreatment conditions were applied on the whole seaweed: control (untreated), freshwater soak, 0.1% KOH, and 0.1 M HCl, —at a sample-to-solution ratio of 1:4 w/v. Biomass were immersed in their respective solutions for <5 min, after which they were weighed to record changes in mass. Then they were mechanically pressed using a twin-screen to separate solid and liquid fractions. Water removal% was calculated based on the initial water content of the post-pretreatment biomass, normalizing for hydration effects to enable accurate comparison of pretreatment efficacy on dewatering. Dry matter (DM), moisture content (MC), and ash content% were determined for both fractions following standard procedures (Milledge and Harvey, 2016).

**Results**

**Figure 1a** reveals notable differences in post-treatment weights and mass variation among treatments in *S.latissima*, especially the higher weight and mass loss variation seen in freshwater soaking and pressing **(SL2)**: Causes strong water uptake, resulting in diluted solids and poorest dewatering performance. Alkali treated **(SL3)**: Also increases water content, but slightly better retention of dry matter than freshwater. Acid treated **(SL4)**: Promotes water loss, yielding higher solid DM% (13.29 DM%) and better dewatering efficiency. SL4 promoted greater liquid release, with SL4 also showing weight loss, possibly reflecting leaching or degradation processes. These outcomes suggest that the treatments alter not only mass balance but also the efficiency of solid–liquid separation and dry matter recovery. While control **(SL1):** Maintains natural balance with relatively high DM% in solid fraction (16.9 DM%) and liquid fraction of 8.7 DM%, serving as reference. Handling loss during pressing was 3.7 to ~8.0% across all treatments but can be brought down further in commercial scale.

**Figure 1b.** The dewatering efficiency of *Alaria esculenta* biomass following different pre-treatment strategies (control, freshwater, KOH, and HCl treatment) was assessed through mass balance analysis of solid and liquid fractions. AE1 (Control): biomass yielding 35.9 g solids and 52.0 g liquid, with a handling loss of 12.1%. The solid fraction exhibited a DM of 28.33%, while the liquid fraction contained 6.39% DM. This reflects efficient retention of dry matter in the pressed cake, serving as the baseline reference for treated samples.

Whereas the biomass gained 24.1% and 49.7% FW when treated with and AE3 (KOH treatment) and AE2 (Freshwater treatment). Handling losses were highest (19.7%), while solid DM% dropped markedly to 23.10% and liquid DM% to 2.51% in AE2 which demonstrates strong dilution of the biomass matrix, producing the poor dewatering performance among treatments compared to AE3.

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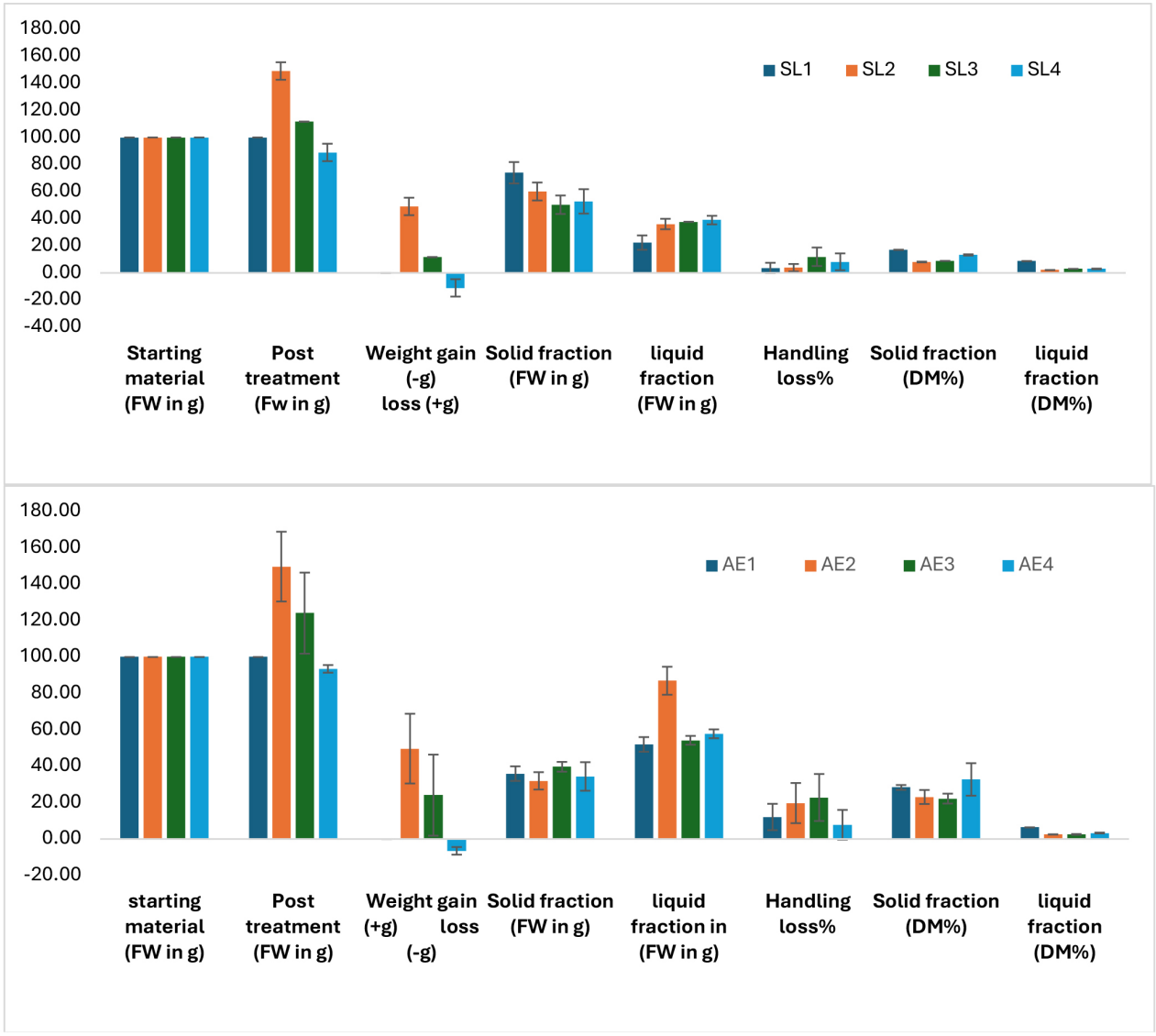


**Figure 1a.** Mass balance of pretreated and pressed *Saccharina latissima* (SL). **Figure 1b**. *Alaria esculenta* (AE) after pretreatment and pressing. The untreated whole biomass contained 13.15 % dry matter (DM) in SL and 20.5 % DM in AE, corresponding to a moisture content (MC) of 86.46 % in SL and 79.49 % in AE.

AE4 (HCl treatment): Biomass lost 6.5% FW, generating 34.4 g solids and 57.8 g liquid with the lowest handling loss (7.83%). The solid DM% increased to 32.73%, while liquid DM% was 3.31%. This treatment significantly enhanced solid concentration and improved water removal efficiency, making HCl soaking the most effective pre-treatment. Among treatments, HCl treated (AE4) was the most effective strategy, yielding the highest solid DM% and lowest handling loss, while freshwater treatment (AE2) was the least effective.

## Conclusion

Overall, acid treated on fresh seaweed found to be promising method to reduce the water content during pretreatment followed by pressing as a dewatering method is best suited both for downstream processing and stabilization of biomass. Whereas untreated seaweed during pressing liberated more dissolved solids and DM% in the liquid fraction. Meanwhile, the liquid fraction was rich in salt content and soluble solids which can be recovered and used for other purposes.

## Acknowledgment

This study as part of the project "AquaUP - Aquafeed Upgraded: Enhancing Immune Function of Fish with Seaweed-modified Functional Compounds" is funded by The Research Council of Norway under Sustainable Blue Economy Partnership (SBEP) co-funded by the European Union.

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# INDUCTION OF POLYPLOIDY IN GREEN ALGA *Ulva fenestrata*: A NEW APPROACH FOR UPSCALING ITS BIOMASS PRODUCTIONS

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**Introduction**

*Ulva fenestrata* is a green seaweed commonly found in Norwegian waters, with strong potential for commercial-scale cultivation. Known as sea lettuce, Ulva spp. are a rich source of protein, vitamins and minerals, and hold strong promise for applications in food, feed, agriculture, bioremediation and biofuel production. Species of Ulva (commonly known as sea lettuce) is a rich source of protein, vitamin and mineral nutrients and it has huge commercial potential in application of food, feed, agriculture, bioremediation and biofuel production field. However, the industry is constrained by the unavailability of high-quality *Ulva* seeds. Polyploidy induction is a widely used technique in agriculture and aquaculture to enhance growth, yield and quality (Fig 1 & 2). Hence, this study aims at developing high vigor polyploid seedlings of *Ulva fenestrata* through treatments with anti-mitotic agents like colchicine and oryzalin.

**Methods**

To achieve this, protoplast isolation and chromosome counting methods are first optimized for this species. Chromosomes are counted in fresh tissue both before and after treatment with anti-mitotic agents at varying concentrations and durations, as well as in protoplasts isolated from these tissues, to assess the effectiveness of the treatments in doubling chromosome numbers via mitotic slippage. DNA content is also measured in isolated protoplasts through flow cytometry (Fig. 3). Once tetraploid *Ulva* cultures are obtained, their growth rate, reproductive traits, biochemical composition, and commercial potential will be compared with those of their wild counterparts. If successful, this approach could open new avenues for up-scaling *Ulva* production.

**Expected results**

- Methodologies to foster *U. fenestrata* breeding and polyploidization:
    - ► High-yield, high-quality protoplast isolation procedure
    - ► Chromosome counting and DNA quantification protocols
    - ► Optimized polyploidy induction method using anti-mitotic agents
- Established polyploid U. fenestrata cultures and sterile and/or fertile seedling production
- Comparative study of growth performance and biochemical composition between polyploid and wild type plants.

# EVALUATION OF THE MICROBIOLOGICAL QUALITY OF FROZEN, DRIED, AND SALTED-DRIED *Octopus vulgaris* : CASE STUDY OF MAHAJANGA

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**Introduction**

The common *Octopus* (*Octopus vulgaris*), also known as the common octopus, is the most commercially fished species of octopus in Europe (Common O., 2025). Conservation and processing techniques also play a crucial role in the distribution and marketing of aquatic products. These techniques include lowering the temperature of products (refrigeration and freezing), applying heat treatment (canning, boiling, and smoking), reducing water content (drying, salting, and smoking), and altering their storage environment (vacuum packaging, modified atmosphere packaging, and refrigeration) (Utilization and Transformation of Fisheries and Aquaculture Production, n.d.). The general objective of this research is to determine which type of conservation treatment is most effective for preservation. To achieve this, it is necessary to assess the effect of different conservation techniques on the microbiological quality of finished products and product stability.

**Materials and Methods**

Fresh octopus specimens were collected from various markets for processing. The specimens were categorized into three samples based on the type of treatment applied: freezing at -20°C for 10 hours, drying in a well-covered solar dryer for 3 to 5 days, and salting and drying. The latter involved immersing the octopus in boiling water for two to five minutes, followed by fermentation with salt for two to three days, and then drying under the sun for 2 to 3 days. Each product was weighed, and the temperature was recorded at each stage and day of processing to ensure treatment effectiveness and evaluate production yield. To ensure the quality of the finished products, the germ count of hygiene indicators such as FTM, total coliforms, *Escherichia coli*, staphylococcus, ASR, yeasts, and molds were performed. Additionally, pathogenic germs (salmonella) were also investigated.

**Results and Discussion**

The results obtained in this study on the microbiological quality evaluation of dried, salted, and frozen octopus showed interesting variations between the different conservation processes. Regarding treatment yield, the frozen octopus displayed high yields, ranging between 90% and 99%. These high yields can be attributed to the freezing process, which better preserves the product's characteristics, particularly by preventing excessive dehydration. In contrast, the salted and dried products showed lower yields (50-64% for salted-dried products and 54-63% for dried products). As (Zakhia, 1992) noted in their research, during 26 hours of drying, the water content of the fish decreased from 77% to 14%. Furthermore, the results from (El Kaouti, 2010) correspond with our findings on salting meat fillets, showing a 20% water loss.

The microbiological analysis of frozen, dried, and salted-dried octopus showed very satisfactory results. All analyzed samples met contamination levels well below critical limits. None of the hygiene indicator germs, such as total coliforms (CT), fecal coliforms (CF), *Escherichia coli* (EC), *Staphylococcus aureus*, and sulfite-reducing anaerobes (ASR), were detected. Additionally, no presence of *Salmonella* was observed. Our results were lower than those recorded by (Hafsa, n.d., 2019). However, we observed the presence of FTM in frozen octopus and a significant observation of molds for the salted-dried sample 2 (50 CFU/g). Nevertheless, this value did not exceed the target limit, indicating that our product was classified as satisfactory. During our experiments, we dried the octopus under the sun, where the temperature ranged between 30°C and 40°C theoretically. **(Zakhia, 1992)** explains that a product dried at 30°C has a risk of *L. monocytogenes* proliferation.

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## Conclusion

Food conservation techniques meet consumer demand. To ensure the effectiveness of these conservation techniques, it is crucial to evaluate the microbiological quality and stability of the product at the end of the process. The three methods chosen (freezing, drying, and salting-drying) for this study are among the most commonly practiced. Overall, our results indicate that freezing remains the easiest technique to master, considering the microbiological quality of the finished products and the yield obtained, although dried and salted-dried products show good results in terms of quality. However, the presence of certain germs, such as FTM, yeasts, and molds, requires improvements, especially in terms of water content and temperature control.

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# OPTIFEESH PROJECT FOR OPTIMIZING FISH PRODUCTION PLANNING: A REAL-WORLD CASE STUDY

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**Introduction**

Optimizing fish feeding is essential to minimize on-farm trial-and-error and robust production planning, enabling aquaculture stakeholders to make informed decisions. Despite increasing digitalization in the aquaculture space and scientific advances on fish nutrition, there is no solution for fish farmers that effectively addresses the major challenge of optimizing feeding planning in aquaculture: The tools available so far do not integrate in-depth knowledge of fish nutrition. It is precisely this gap that OptiFeeSH project aims to fill by combining technical know-how in nutrition with digital intelligence, offering an innovative solution geared towards making informed and efficient decisions.

**OptiFeeSH approach**

The development of OptiFeeSH is driven by expertise in fish production and industrial R&D, integrating fish nutrition and farming technology within a user-friendly interface. Its Optimization module applies a biological approach based on a nutritional-based mathematical model, calibrated by species and sensitive to the feed energy and protein content. In a first step the system calculates four reference base plans:

- Maximum growth plan: feeding rates that ensure maximum growth.
- Lowest FCR plan: feeding rates that ensure the lowest FCR.
- Maximum profit plan: feeding rates that ensure the maximum profit.
- Meet requirements: fish feed requirements to ensure the user target weight.

In the second step, the engine creates interpolated plans, delivering a tailored set of feeding strategies aligned with the user's specific production goals (Table 1).

**Case study for a seabream cage farming**

Figure 1 shows a subset of the plans generated by OptiFeeSH for the bream case study (Table 1), which allows users to evaluate and choose according to their optimization targets: maximize profit, maximize growth, minimize FCR. All OptiFeeSH plans forecast improved profit and FCR compared to the current user plan (Figure 1). The Meet Requirements Plan, designed to meet nutritional needs for the user input target weight and production period, aligns closely with real production data (Figure 2) and as shown in Figure 1, outperforms current user plans in both profit (€0.84 vs. €0.64 per kg biomass) and economic FCR (1.69 vs. 1.83). Plan 8 delivers the fastest growth, while Plan 3 yields the highest profit for a 150,000-fish cage. Plan 3 provides a different feeding strategy, by increasing feeding rates for fish bigger than 250g, when comparing with the Meet Requirements, reaching the 450g target, 11 days earlier and increasing the profit of the cage from 50.9k € to 51.5 k €. The subset of plans listed in Figure 1 also shows that a lower economic FCR does not always translate to higher profitability.

**Conclusion**

OptiFeeSH provides a sound scientific approach to create multiple production plans that meet each farm target growth and allows the user to choose the optimal one according to their criteria: faster growth, minimum FCR, maximum profit, or a compromised solution. These tailored feeding and production plans help avoid overfeeding, fine-tune FCRs, and detect underfeeding early, providing a solid basis for daily feeding decisions.

This work is part of the E!2498 OptiFeeSH project, No. 17506, Operation No. MPr-2023-4-17506 (COMPETE2030-FEDER-01004100, ALGARVE-FEDER-01004100), supported by the EUROSTARS-3 program and co-financed by the European Union through the Algarve 2030 and COMPETE 2030 Operational Programs, in the framework of Portugal 2030, RESTART 2016-2020 Programme (Cyprus), and Participation of Greece in two calls for proposals of the co-funded European Partnership for Innovative SMEs of Horizon Europe.

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Table 1. OptiFeeSH input data for a real-world bream production scenario, based on the HAPPO (2023).

| | |
|---|---|
| **Temperature Profile** | 15º C to 29º C |
| **SFR and FCR tables** | User current plan |
| **Initial Fish No** | 150 000 |
| **Initial Av. Weight (g)** | 2.5 |
| **Stocking Date** | 01/10/2023 |
| **Mortality Rate for Target Period (%)** | 10 |
| **Target Av. Weight (gr)** | 450 |
| **Target Date** | 01/12/2024 |
| **Fish Price** | 4.8 |
| **Fry Cost** | 0.2 |
| **Monthly General Expenses** | 4 000 |

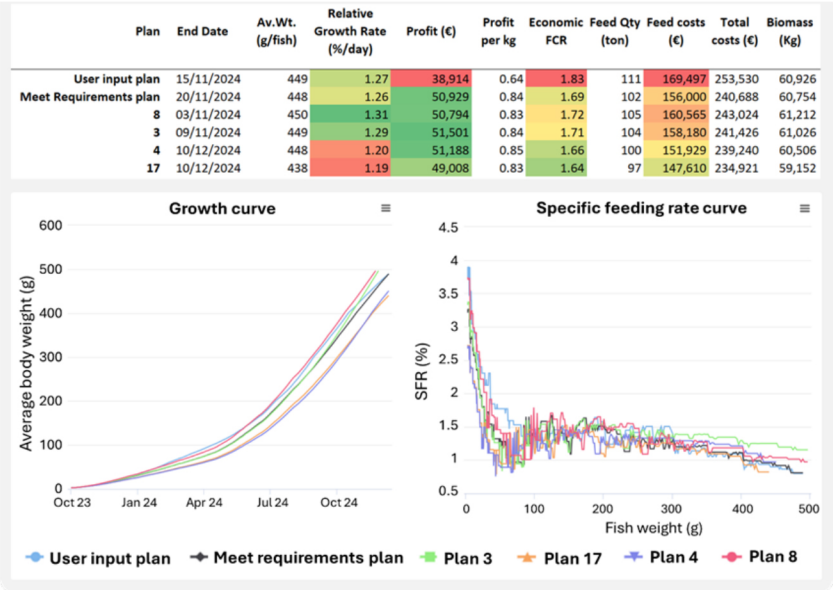


Figure 1. OptiFeeSH results, subset of the plans generated for the bream case study: summary table with KPIs for the production cycle, growth curves and feeding rates.

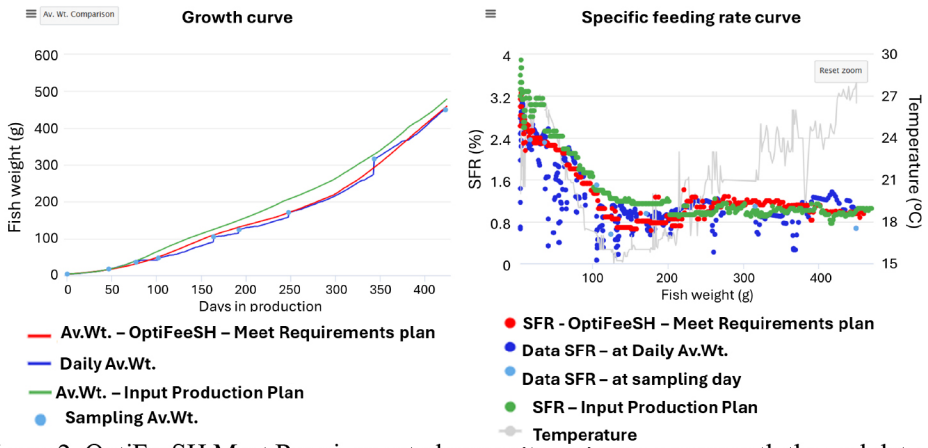


Figure 2. OptiFeeSH Meet Requirement plan results and comparison with the real data.

# FEEDNEXT: ADVANCING DIGITAL INNOVATION AND SUSTAINABILITY IN MEDITERRANEAN AQUACULTURE THROUGH TRAINING AND CAPACITY BUILDING

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**Introduction**

The FEEDNEXT initiative addresses the need for digital innovation and capacity building within Mediterranean aquaculture by equipping students, young professionals, unemployed workers, and industry stakeholders with essential skills in precision feeding, digital tools, and sustainable aquaculture practices. Implemented across Portugal, Greece, Tunisia, Turkey, and Egypt, the initiative fosters equal access to training through development of interactive learning modules, online resources, and practical applications that align with the green and digital transitions. By leveraging the expertise of research institutions, universities, and industry partners, FEEDNEXT aspires to establish a transnational platform to strengthen digital literacy and sustainable aquaculture practices. The initiative is dedicated to delivering scalable and transferable educational content that supports long-term skills development and sustainability in aquaculture.

**Methods and implementation**

FEEDNEXT aims to implement a capacity-building initiative that will address essential subjects like data digitization, aquaculture nutrition, precision feeding strategies, and circular economy principles, including Life Cycle Assessment (LCA) in aquaculture. The training materials will be translated into the languages of partner countries and made accessible via a Moodle platform, ensuring accessibility for a broad audience. The project will also create pilot actions designed to function as interactive training platforms, allowing participants to gain practical experience analyzing real-world data, applying advanced tools, and addressing sector-specific challenges. The training modules will also include practical tutorials with FEEDNETICS, allowing participants to gain experience on digital prediction tools. FEEDNEXT aims to equip the participants with the digital and technical skills necessary for sustainable aquaculture.

**Expected outcomes and impact**

FEEDNEXT expects to foster a new generation of aquaculture professionals by bridging the gap between research, industry, and education. The initiative aims to enhance digital skills, precision feeding knowledge, and sustainable practices/methods, equipping students, young professionals, and unemployed workers with the necessary tools for a greener and efficient aquaculture sector. By advocating for circular economy concepts, resource-efficient fish farming, and digital literacy, FEEDNEXT will contribute to the EU Green Deal objectives and the UN Sustainable Development Goals. Through capacity-building initiatives, e-learning materials, and hands-on training, the program will contribute to improving employability and strengthen industry-academia collaboration. FEEDNEXT will ensure its long-term scalability and adaptability by partnering with universities, research institutes, and industry stakeholders throughout the Mediterranean.

**Conclusion**

By integrating digital innovation, sustainability, and education, FEEDNEXT aims to strengthen the resilience and competitiveness of Mediterranean aquaculture. The initiative's approach will not only enhance skills development and employment opportunities but also supports the transition toward a more sustainable and eco-friendly blue economy.

# BACTERICIDAL AND BACTERIOSTATIC ACTIVITIES OF HALOPHYTE BASED BIOACTIVE COMPOUNDS AGAINST BACTERIAL PATHOGENS

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## Introduction

The modern expansion of aquaculture is frequently accompanied by the emergence of diseases, leading to significant impacts on the production of various fish species, including the economically important European seabass (*Dicentrarchus labrax*). To address this challenge, and to combat antimicrobial resistance due to irresponsible and inappropriate use of antibiotics, exploring sustainable and eco-friendly strategies could have great potential for the industry. Bioactive compounds from halophytes have recently gained significant attention for use in the nutraceutical, pharmaceutical, and cosmetic industries. Phytochemicals including natural compounds, secondary metabolites, and their derivatives can play active roles in antibacterial, anti-inflammatory, and antioxidant activities (Giordano *et al*., 2021). This study aimed to evaluate the antimicrobial potential of bioactive compounds from *Salicornia ramosissima* residual biomass for applications in aquaculture while applying circular economy concepts.

## Materials and methods

The bactericidal and bacteriostatic potential of seven extracts (Ext1, Ext2, Ext3, XAD, XAD3, FERM, FRAC3) obtained from the halophyte *S. ramosissima* were tested at 0.125, 0.25, and 0.5 mg·mL$^{-1}$ against bacterial pathogens for fish and shrimp: *Aeromonas hydrophila, Edwardsiella piscicida, Photobacterium damselae piscicida, Tenacibaculum maritimum, Vibrio anguillarum, Vibrio parahaemolyticus, and Yersinia ruckeri*. The bactericidal activity of the extracts was determined by a colorimetric MTT assay (Machado *et al*., 2024) and expressed as % viability inhibition, while the bacteriostatic activity was measured as % growth inhibition at the 24-h endpoint, using $OD_{630}$ recorded at 13 time points from 0–24 h (Mai *et al*., 2023)

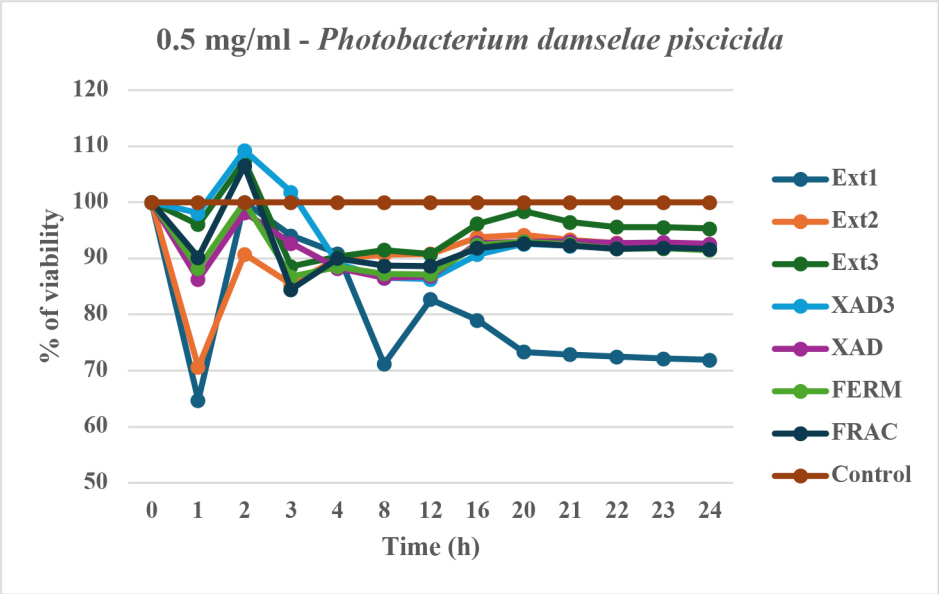


Figure 1. Bacteriostatic activity of extracts against *Photobacterium damselae piscicida* at 0.5mg/ml

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**Results**

Ext1, Ext2, Ext3, XAD, and FRAC3 showed modest bactericidal activity (31.42–40.55%) at the highest concentration (0.5 $mg \cdot mL^{-1}$) against *P. damselae piscicida* and *Y. ruckeri*, while XAD and FERM showed mild viability reductions (17.63–18.65%) against *T. maritimum*. In contrast, almost all extracts showed very weak to no inhibition of viability against *A. hydrophila, E. piscicida, V. anguillarum, and V. parahaemolyticus* at all concentrations tested. Overall, while most tested *S. ramosissima* extracts displayed a narrow-spectrum bacteriostatic profile, Ext1 showed a moderate bacteriostatic activity against *P. damselae piscicida*, which translates into aprox. 29% inhibition at concentration 0.5 $mg \cdot mL^{-1}$ (Figure1)

**Conclusion**

In conclusion, these findings indicate that some extracts could act as promising antibacterial agents. However, further studies are needed to increase the concentration range to examine dose–response/$IC_{50}$, as well as confirming killing via Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC) or colony forming unit counts and assess potential synergies. Also, its therapeutic or prophylactic effect in vivo should be addressed to evaluate their potential as nutraceutical ingredient.

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**Acknowledgements**

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# THE EFFECTS OF EPINEPHRINE AND GABA AS CHEMICAL INDUCERS OF METAMORPHOSIS IN *Ruditapes decussatus* AND *Venerupis corrugata*

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## Introduction

Bivalves have a complex life cycle, encompassing a planktonic free-swimming larvae and a benthonic phase[1]. Metamorphosis is responsible for the transition from larvae to the juvenile stage and represents a critical step in the bivalve life cycle. This process is one of the most challenging phases for bivalve hatcheries due to high mortality and a non-synchronous larval development within a cohort – not all the individuals are physiologically competent to metamorphose at the same time[2]. To achieve a greater, more rapid and efficient percentage of metamorphosis and consequently improve the production[3,4], hatcheries could use synthetic chemical inducers. Among the compounds with an inductive effect on metamorphosis, the amino acid derivative γ-aminobutyric acid (GABA) and the catecholamine epinephrine (EPI) are considered as the most efficient.

Grooved carpet shell (*Ruditapes decussatus*) and Pullet carpet shell (*Venerupis corrugata*) are species with high commercial and ecological importance, widely distributed in coastal and estuarine waters throughout Europe, the Mediterranean and North Africa[5–7]. In Portugal, while *R.* decussatus, which is produced and harvested mainly in Ria Formosa lagoon, is crucial for aquaculture revenue (representing 90% of the national production), *V. corrugata* is emerging as a relevant species to Portuguese bivalve aquaculture. Considering the relevance of these species and, in order to improve metamorphosis, with reduced mortality in bivalve hatcheries, the current work evaluated the effects of GABA and EPI, in terms of optimal concentrations, toxicity and exposure time.

## Material and methods

Larvae were exposed to three different concentrations ($10^{-2}$, $10^{-4}$ and $10^{-6}$ M) of EPI and GABA and filtered sea water (FSW) was used as control. EPI and GABA stock solutions were initially prepared at a concentration of $10^{-1}$ M and $10^{-3}$ M; stock solutions were then diluted in FSW to reach the experimental concentrations immediately before experiments.

Metamorphosis trials were conducted in glass beakers with 300 ml of FSW, under continuous aeration. Each beaker contained a sieve (150 $\mu$m mesh) with approximately 1000 larvae (3 larvae $mL^{-1}$). Larvae were exposed to different concentrations for 24, 48 and 72 hours. After the exposure period, larvae were rinsed with FSW and 3 aliquots with a minimum of 30 larvae of each beaker were analysed, to evaluate mortality and metamorphic stages. The developmental stages were characterized by the presence of velum, foot and velum, foot, or foot and branchial arches. A larva was considered to have undergone complete metamorphosis when it had lost its velum, was using its foot to crawl and had developed branchial arches. After evaluation, larvae were placed back into the beakers with clean FSW for an additional period of 5, 4 and 3 days, respectively. Metamorphosis rate and mortality were evaluated after each exposure time and at day 6 after the onset of exposure.

## Results and Discussion

Figure 1 shows the effects of EPI and GABA on the metamorphosis rate of *V. corrugata* and *R. decussatus* after each exposure time. In general, high concentrations of both EPI and GABA ($10^{-2}$ M and $10^{-4}$ M) revealed to be toxic to the larvae of both species, regardless of the exposure time (fig. 2). The higher metamorphic rate in *V. corrugata* and lower mortality were achieved after exposure to EPI $10^{-6}$ M (24h and 48h) and GABA $10^{-6}$ M (48h) (fig. 1). At 6$^{th}$ day, the 48h-exposure to GABA and EPI $10^{-6}$ M induced a higher metamorphosis rate, varying between 62.2% and 82.2%, respectively. Despite its effectiveness in inducing metamorphosis, *V. corrugata* appeared to be more sensitive to EPI, exhibiting higher mortality rates at certain concentrations.

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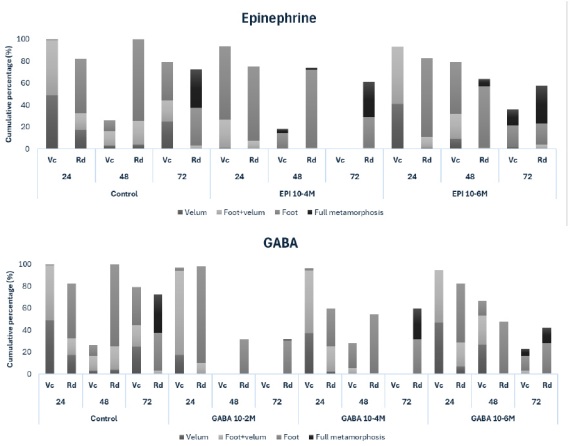


**Figure 1-** Metamorphic induction after exposure (24h, 48h and 72h) to epinephrine and GABA on larvae of *Venerupis corrugata* (Vc) and *Ruditapes decussatus* (Rd).

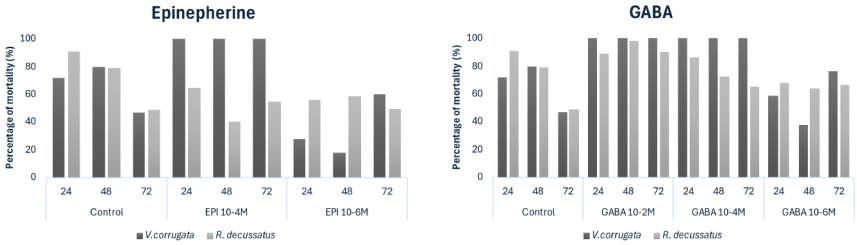


**Figure 2-** Percentage of mortality of larvae of *Venerupis corrugata* and *Ruditapes decussatus* observed at the end of the trial (6$^{th}$ day) after exposure to epinephrine and GABA.

In contrast, GABA failed to induce metamorphosis in *R. decussatus*, besides being toxic to the larvae. For this species, 72h-exposure to EPI $10^{-4}$ M and $10^{-6}$ M induced a higher metamorphic rate, varying between 32.2% and 34.3%, respectively (fig.1). At 6$^{th}$ day of the trial, metamorphosis reached 55.6% and 50.5% in larvae exposed to EPI $10^{-4}$ M (48h) and $10^{-6}$ M (72h).

These findings highlight the species-specific responses to chemical inducers. Optimal concentration and exposure time vary between species, emphasizing the need to adapt conditions accordingly. Adjusting these parameters may enhance hatchery production by improving metamorphosis efficiency while minimizing larval mortality.

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# INTEGRATED MULTI-TROPHIC AQUACULTURE IN THE ATLANTIC: IS IMTA THE ANSWER FOR SUSTAINABLE, RESILIENT AND PROFITABLE AQUATIC VALUE CHAINS? FINAL RESULTS FROM THE ASTRAL PROJECT

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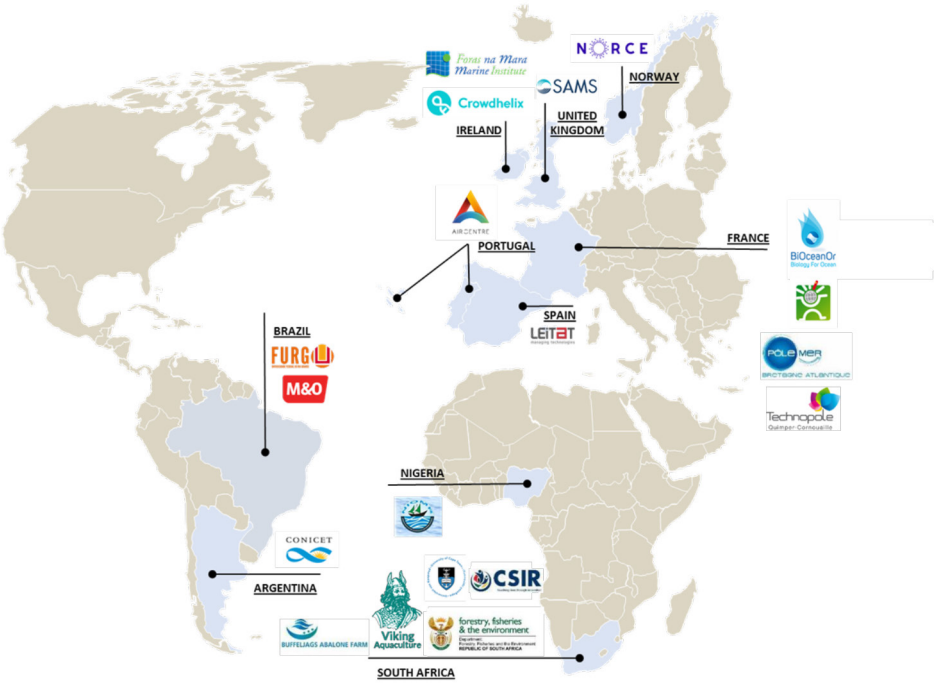
The EU-funded ASTRAL project has developed IMTA production chains for Atlantic markets. Focusing on a regional challenge-based perspective, it brought together labs in Ireland and Scotland (open offshore labs), South Africa (flow-through inshore, partially recirculating) and Brazil (recirculation inshore) as well as Argentina (prospective IMTA). The aim has been to increase circularity compared to monoculture baseline aquaculture and to boost revenue diversification for aquaculture producers. The ASTRAL project has also fostered a collaborative ecosystem along the Atlantic through shared, integrated, and co-generated knowledge, technology and best practices.

The conclusive results on increased circularity, best practices for zero waste, economic and social assessments will be presented.

Acknowledgment: The ASTRAL project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 863034.



# EVALUATION OF THE ANTIVIRAL ACTIVITY OF NATURAL SUBSTANCES OBTAINED FROM BACTERIA AND ALGAE AGAINST VIRAL HAEMORRHAGIC SEPTICEMIA VIRUS

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## Introduction

Viral haemorrhagic septicaemia virus (VHSV) infects a wide range of freshwater and marine fish species, causing high mortality rates in susceptible cultured fish, mainly salmonids (Smail and Snow, 2011). A prophylactic practise to enhance the natural resistance of farmed fish to viral infections is the use of functional food. Extracellular polymers (ECPs) from probiotic bacteria are postbiotics that represent a valid and safe alternative for providing beneficial effects against microorganisms in fish (Domínguez-Maqueda *et al.*, 2024; García-Márquez *et al.*, 2023). Macroalgae and microalgae are also alternative sources of substances with potential activity to improve resistance to microbial infections (Abdala-Díaz *et al.*, 2021; Parra-Riofrio *et al.*, 2023). The objective of this study was to evaluate the antiviral activity of ECPs from the probiotic bacteria *Shewanella putrefaciens* Pdp11 and *Bacillus pumilus* UMA169, as well as extracts and/or polysaccharides from *Ulva rigida* and *Porphyridium cruentum*, against a freshwater VHSV isolate.

## Materials and methods

The protection conferred by each substance against a freshwater VHSV isolate (genotype 1) was evaluated *in vitro*, on RTG-2 cells, by quantifying the cellular survival rate after viral infection, using the MTT reduction assay. Cells were inoculated at 0.05 MOI, and, after an adsorption time of 1 hour, the viral suspension was removed, and replaced by cultured medium supplemented with the substances. Cells were cultured at the optimum temperature for viral replication, and the cellular survival rate was calculated 3 days post-infection. The substances tested were: (i) ECPs from *B. pumilus* UMA169, ECPs from S. *putrefaciens* Pdp11 (both bacteria cultured on TSAs at 15 ºC for 48 h) at 50 µg of protein·mL$^{-1}$, (ii) extracts of *U. rigida* at 100 and 500 µg·mL$^{-1}$, (iii) ulvan polysaccharides from *U. rigida* at 50 µg·mL$^{-1}$ and (iii) extracts of *P. cruentum* at 100 µg·mL$^{-1}$.

## Results

All substances tested exhibited antiviral activity against VHSV, except for ECPs from Pdp11, which showed the opposite effect (Figure 1). ECPs from *B. pumilus* UMA169 conferred full protection to the cells, whereas algal extracts and ulvan polysaccharides conferred a similar protection level to VHSV infected cells. Thus, these substances, excluding ECPs from Pdp11, are promising candidates to be used as protective substances against VHSV. Actually, their effect on viral replication and infective viral particles production will also be evaluated.

According to these results, a supplemented diet will be designed to test the anti-VHSV activity of these substances in the experimental model of zebrafish to evaluate their antiviral potential and relevance to aquaculture.

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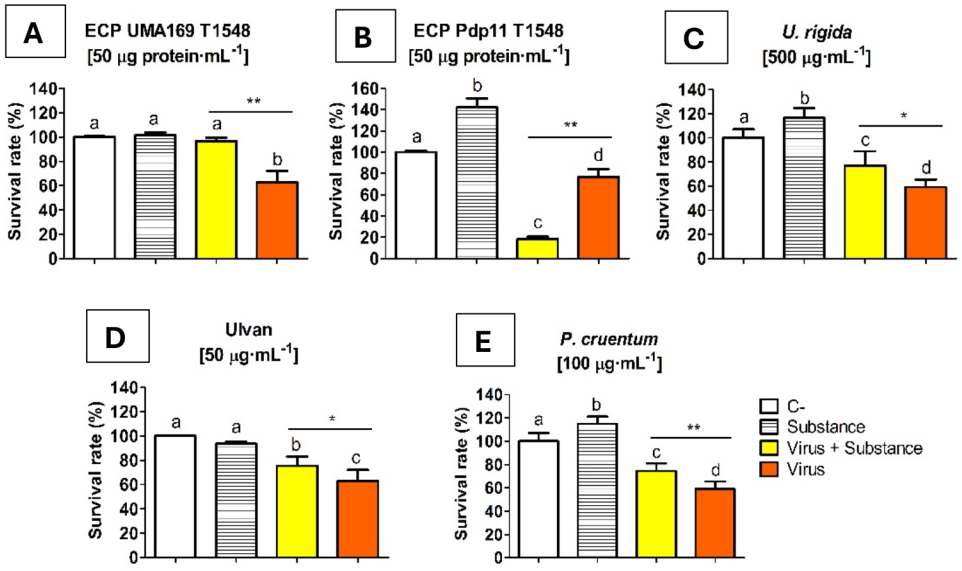


**Figure 1 :** Survival of RTG-2 cells exposed to ECP UMA169 (T1548) (A), ECP Pdp11 (T1548) (B), *U. rigida* extracts (C), ulvans (D) and *P. cruentum* extracts (E). Cells were inoculated with a freshwater VHSV isolate at 0.05 MOI. The survival rate was evaluated at 3 days post-infection. Data were statistically analysed with the two-way ANOVA test. Different letters denote significant differences between groups. Values of $P < 0.01$ (*) or $P < 0.001$ (**) were considered significant. Results are mean ± standard deviation (SD) (n = 8).

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# INMUNOSTIMULATORY AND ANTIVIRAL ACTIVITIes OF TWO MARINE AMPHIPODS IN FISH CELL LINES

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## Introduction

One of the main priorities for the aquaculture industry is the development and implementation of measures to prevent viral diseases. In this regard, the design of functional food is taking great relevance, since it may stimulate the unspecific antiviral immune system, protecting fish from different viral infections. The main unspecific antiviral immune system is the interferon I (IFN-I) system, responsible for the induction of hundreds of IFN-stimulated genes (ISGs), such as *mx*. The objective of this study was the *in vitro* evaluation of the immunostimulatory and antiviral activities of two marine amphipod species (*Caprella scaura* and *Gammarus insensibilis*), which have high nutritional value and great potential as new trophic resource in aquaculture (Guerra-García *et al*., 2016; Jiménez-Prada *et al*., 2018)

## Materials and methods

The stimulation of the IFN-I system was evaluated using two celular systems: (i) RTG-2 cells expressing luciferase under the control of the Senegalese sole (*Solea senegalensis*) *mx* promoter (p*ssmx*, RTG-2-p*ssmx-luciferase system*), and (ii) RTG-2 cells expressing luciferase under the control of the gilthead seabream (*Sparus aurata*) *mx2* promoter (p*saumx2*, RTG-2-p*saumx2-luciferase system*) (Alvarez-Torres *et al*., 2013; González-Mariscal *et al*., 2014). These cells were exposed to different concentrations of lyophilized amphipods during several periods, and subsequently washed, lysed and mixed with the luciferase substrate. Stimulation of p*ssmx* and/or p*saumx2* promoters was quantified as luciferase activity, which was measured as relative light units. Inducibility was calculated according Alvarez-Torres *et al.* (2013). The antiviral activity was evaluated against a marine viral haemorrhagic septicaemia virus (VHSV) isolate (genotype 3) on RTG-2 cells following three procedures: (i) viral neutralization assay, (ii) 1-h pre-adsorption assay, and (iii) post-adsorption assay (Moreno *et al*., 2023). Results were expressed as cellular survival rate, which was calculated 3 days after the viral infection by the MTT-reduction assay.

## Results

The Senegalese sole *mx* promoter was not induced by any of the amphipods tested, however both species induced gilthead seabream *mx2* promoter. Specifically, p*saumx2* was induced at 3 days after cell exposure to *G. insensibilis* (100 $\mu$M) and, at 3 and 4 days after *C. scaura* exposure (100 and 250 $\mu$M) (**Figure 1**). Nevertheless, only cells exposed to *G. insensibilis* (100 and 250 $\mu$M) after VHSV infection (post-adsorption assay) exhibited survival rates significantly higher than those obtained for the control non-exposed cells (**Figure 2**).

We can conclude that both species are able to activate the IFN-I signalling pathway in RTG-2 cells, although only *G. insensibilis* provides *in vitro* protection against VHSV. In consequence, these two species are promising candidates to be used as immunostimulants in aquaculture, and they may induce an antiviral state in the host, which probably is species dependent.

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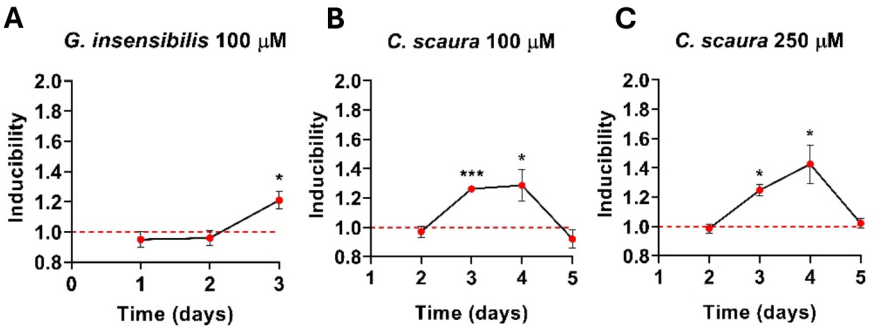


**Figure 1:** Inducibility of the RTG-2-p*saumx2-luciferase* system by exposure to *G. insensibilis* at 100 µM (A) or to *C. scaura* at 100 (B) and 250 (C) µM. Dashed-red line is the threshold of inducibility. Data were analysed with the Student's t-test. Asterisk denotes significant induction ($P$<0.05 *; $P$<0.001 ***). Results are mean ± standard deviation (SD) (n=3).

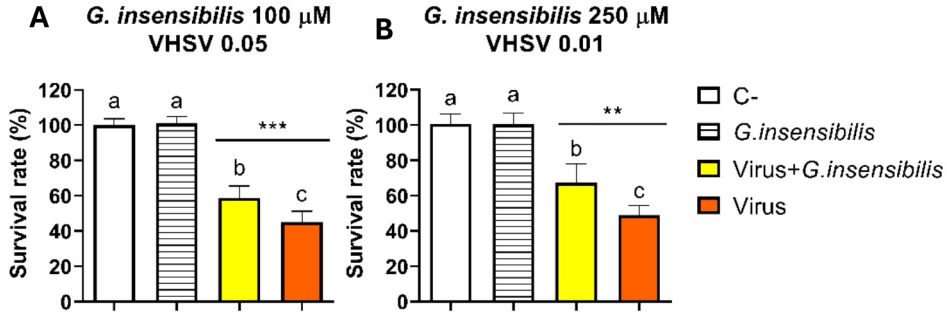


**Figure 2:** Survival of RTG-2 cells exposed to *G. insensibilis* at 100 (A) and 250 (B) µM after viral infection. Cells were inoculated with a marine VHSV isolate at 0.05 (A) and 0.01 (B) MOI. Data were analysed with the two-way ANOVA test. Different letters denote significant differences. Values of $P$<0.01(**) or $P$<0.001(***) were considered significant. Results are mean ± standard deviation (SD) (n=8).

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# INVESTIGATING THE POTENTIAL OF DIETARY BETA-GLUCANS FROM *Saccharomyces cerevisiae* TO MITIGATE ACUTE INDUCED COLITIS IN ZEBRAFISH (*Danio rerio*)

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Maintaining gut health is essential for optimal growth, feed efficiency, and disease resistance in aquaculture species. Inflammatory bowel conditions, whether caused by pathogens, diet, or environmental stressors, represent a major threat in intensive aquaculture systems. This study aims to investigate the effects of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in zebrafish as an *in-vivo* model for acute intestinal inflammation and assess the potential mitigating effects of dietary beta-glucans, as shown in Figure 1.

Beta-glucans are complex polysaccharides derived from yeast and other fungal cell walls. They are known immunomodulators and have demonstrated anti-inflammatory properties in various animal models. Beta-glucans can increase macrophage functionality and mobilise other immune cells including natural killer cells, which play a crucial role in controlling inflammation and infection. We hypothesize that dietary supplementation with beta-glucans can reduce TNBS-induced inflammation in the posterior intestine of zebrafish, potentially by modulating innate cellular responses and preserve intestinal integrity.

This model will serve as a foundation for developing functional feeds that support intestinal health in commercially important aquaculture species. Results from this research may contribute to sustainable health management strategies in fish farming.

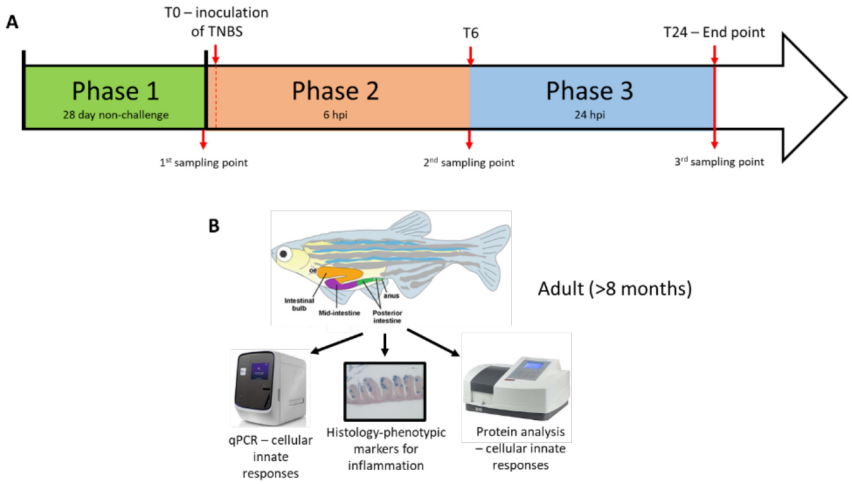


Figure 1. Schematic showing the trial (A) experimental design and (B) sampling endpoints. Figure B shows in green the posterior intestine, this intestinal region was sampled for analysis.

# GILLS, GENES AND GRADIENTS: INVESTIGATING SALINITY TOLERANCE IN MARAENA WHITEFISH (*Coregonus maraena*)

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## Introduction

Fish employ a range of physiological mechanisms to acclimate to environmental salinity levels that differ from their internal osmotic conditions. Many of these adaptive responses are mediated by characteristic, teleost-specific gene expression patterns. Among the most studied are two isoforms of the *atp1a1* gene, which encode the α-subunit of the Na□/K□-ATPase, an essential enzyme for osmoregulation. These isoforms have become established transcriptional markers in aquaculture, particularly for predicting the seawater adaptability of Atlantic salmon *Salmo salar* (McGowan et al., 2021).

Despite the growing body of research on osmoregulatory markers in salmonids, transcriptional indicators of saltwater tolerance in coregonids remain largely unexplored. To address this gap, we profiled gene expression in selected tissues of maraena whitefish *Coregonus maraena* reared either in the Baltic Sea with naturally fluctuating salinities or in a freshwater facility (see Figure a). Acknowledging the influence of variable environmental conditions in these two open aquaculture systems, we also conducted a complementary salinity challenge under standardised conditions in a recirculating aquaculture system (RAS).

## Material and methods

To investigate the potential effects of salinity on the expression of genes associated with salinity adaptation, we analysed transcript levels in the gills, skin, head kidney, and liver of maraena whitefish over a 10-week period. Fish were reared either in a freshwater pond system (BiMES, Friedrichsruhe/Frauenmark, Germany) or in a net-cage facility located within an artificial reef in the Baltic Sea with fluctuating salinity levels between 8.6 and 12.0 PSU (see Figure a). To complement field data and account for potential confounding environmental variables in the open systems, a controlled follow-up experiment was conducted at the experimental aquaculture facility of the FBN (Dummerstorf, Germany). During this 9-day experiment, two RASs were used: one maintained with freshwater, and the other adjusted to 8 PSU and later to 20 PSU for 48 h, using sea salt (Sale Marino, LP20259B, Fino Margherita, Milano, Italy) (see Figure b).

Gene expression profiling was performed using the BioMark HD system (Standard BioTools, San Francisco, CA, USA). A total of 23 candidate genes associated with salinity tolerance, along with two reference genes, were selected based on prior work by Houde et al. (2019).

## Results and discussion

The *atp1a1a* isoform showed significantly higher expression in the gills of maraena whitefish reared in the freshwater pond compared to individuals from the brackish-water reef. In contrast, the seawater-associated isoform *atp1a1b* was significantly upregulated in the gills of whitefish from the reef relative to their pond-reared conspecifics (Figure c). Despite these differences, the overall expression profiles between the two groups were largely comparable. A PCA based on gene expression across all sampled tissues did not reveal clear separation between reef- and pond-reared individuals, indicating a high degree of similarity in their transcriptional profiles (Figure d). In the gills, as in the three other tissues analysed, only sporadic modulations in transcript abundances across time points were observed (Figures e and f).

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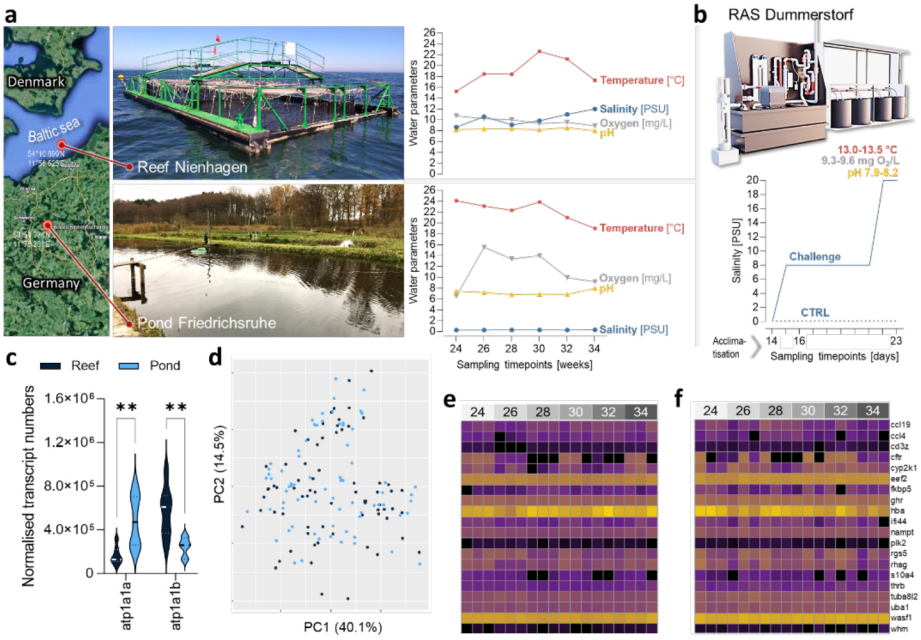


**Figure: Overview of experimental sites, environmental conditions and gene expression analyses in maraena whitefish.** (**a**) Map section (adapted from Google Maps) showing the locations of the two whitefish production facilities: the brackish-water reef (top left) and the freshwater pond system (bottom left). Temperature (red), salinity (blue), oxygen concentration (grey), and pH (yellow) are plotted over the 10-week observation period. (**b**) Schematic representation of the RAS (top) used for the 9-day salinity treatment illustrated over time (bottom). (**c**) Violin plots showing normalised expression levels of *atp1a1a* and *atp1a1b* in the gills of whitefish reared under reef (dark blue) and pond (light blue) conditions (n = 7). Asterisks indicate statistically significant differences (adjusted p = 0.005). (**d**) PCA plot showing the separation of gene expression profiles from whitefish reared in the reef (dark blue) and pond (light blue). (**e–f**) Heat maps of log10-transformed transcript counts for selected genes in gill tissue of whitefish from (**e**) the reef and (**f**) pond systems (n = 3 per condition). Sampling time is shown above each heat map; gene symbols are listed on the right.

In the follow-up experiment conducted in the RAS, an increase in salinity from 0 to 8 PSU led to significant decreased *atp1a1a* and increased *fkbp5* transcript levels in the gills. Elevation to 20 PSU was associated with further reduced levels of *atp1a1a* in the gills.

Overall, the data suggest that maraena whitefish tolerate salinities up to 20 PSU without showing signs of physiological stress. However, the limited transcriptional differentiation observed under natural rearing conditions indicates that the current set of candidate biomarkers may be less effective for evaluating open aquaculture systems. In contrast, gene expression responses in the RAS were more consistent and pronounced, highlighting the potential of these markers for controlled-environment applications.

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# CLIMATE CHANGE ADAPTATIONS IN SALMON AQUACTULRUE: ECOSYSTEM SERVICE IMPACTS AND TRADE-OFFS

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Marine aquaculture systems rely on services provided by the natural environment to support food production, but the marine environment and the ecosystem services it provides are increasingly affected by climate change. Climate change impacts including decreased oxygen levels, heat waves, increasing incidence of jellyfish and algal blooms, and extreme weather events and storm surges are affecting aquaculture production, fish health, and the welfare of farmed species. Coping strategies, transformational change, and technological solutions may allow farmers and companies to adapt to climate change, but the impact of these adaptations is unknown. We are developing a Bayesian Belief Model (BBN) based on expert elicitation to explore the impacts of climate change adaptations on ecosystem services. Using marine salmon farming as an example, this approach provides a means to explore potential aquaculture futures and identify trade-offs between ecosystem services under different climate change and adaptation scenarios.

**Acknowledgements**
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# EFFECT OF POMEGRANATE EXTRACT SUPPLEMENTATION ON GROWTH AND INTESTINAL MICROBIOTA OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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## Introduction

Additives are widely used in aquaculture as proactive nutritional strategies to improve fish nutrient utilization and health, with some of their benefits potentially related to changes in intestinal microbiota composition and function. The intestinal microbiota plays region-specific roles in the digestion and synthesis of key metabolites, such as short-chain fatty acids (SCFAs), amino acids, and vitamins [1]. Pomegranate (*Punica granatum*), a fruit rich in polyphenols such as punicalagin, has shown functional potential by modulating microbial communities, suppressing pathogens, promoting commensals, and stimulating SCFAs synthesis [2]. Several studies have demonstrated that pomegranate-derived additives enhance the growth, immunity, and disease resistance of rainbow trout (*Oncorhynchus mykiss*) [3], rohu (*Labeo Rohita*) [4]it was observed that pomegranate peel ethanolic extract (PPEE, common carp (*Cyprinus carpio*) [5], and Nile tilapia (*Oreochromis niloticus*) [6]. However, no data are available on their effects on European sea bass (*Dicentrarchus labrax*), especially regarding the intestinal microbiota, using next-generation sequencing (NGS). This study aimed to evaluate the effect of pomegranate extract (PM) on the growth and intestinal microbiota of European sea bass juveniles, as a tool to promote productivity and microbial stability.

## Materials and methods

Juvenile sea bass (n = 200; 12.71 ± 0.08 g) were randomly distributed into six 0.5 m³ tanks (3 replicates per diet) in IRTAmar™ recirculation aquaculture systems (RAS). Fish were fed either a control diet (CTRL) or a pomegranate-supplemented diet (PM; 0.02%) for 12 weeks. At the end of the feeding trial, fish were individually weighed, and 12 fish per tank were sampled to collect the anterior (AI) and posterior intestine (PI) mucosa for microbiota analyses. DNA was extracted using the DNeasy® PowerSoil® Pro Kit, and the V3–V4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq (2 × 300 bp). Sequence processing and taxonomic classification were performed in R using QIIME2, DADA2, and Microbiome packages and SILVA v138.1 database. Diversity indices were calculated using the microeco package. Statistical analyses included LMM for alpha diversity, PERMANOVA for beta diversity, and ANCOM-BC for differential abundance ($p \leq 0.05$), with diet and intestinal region as variables. For growth performance, a t-test ($p \leq 0.05$) was conducted after verifying the normality and homogeneity of variances.

## Results and discussion

After 12 weeks of dietary supplementation, PM dietary supplementation did not significantly affect growth performance ($p > 0.05$; average SGR of 1.93 ± 0.05%). According to the bioinformatic results, a total of 8.9 million clean reads and 895 ASVs were obtained. No significant differences were observed in alpha or beta diversity across diets or intestinal regions (LMM and PERMANOVA; $p > 0.05$). However, differential abundance analysis (ANCOM-BC; $p \leq 0.05$) revealed an effect of PM supplementation on 2 phyla and 22 genera; of intestinal region on 3 genera; and of the interaction of both variables on 1 phylum and 23 genera. Subsequent analyses stratified by intestinal region showed that in the AI, the dominant phyla (>5% relative abundance) were Proteobacteria, Actinobacteriota, and Firmicutes. Halobacterota, mainly represented by *Haloccocus*, was significantly enriched in fish fed the PM diet. Dominant genera included *Pelomonas*, *Cutibacterium, Corynebacterium, Staphylococcus, Vibrio,* and *Shewanella.* PM supplementation also increased the moderately abundant (1–5% relative abundance) *Planctomicrobium* and low-abundance (<1% relative abundance) genera such as *Francisella, Delftia,* and *Caulobacter*, while *Coxiella* was higher in fish fed the CTRL diet. In the PI, dominant phyla included Proteobacteria, Actinobacteriota, Firmicutes, Chloroflexi, and Bacteroidota. Fish fed the PM diet presented increased Halobacterota (*Halococcus*), as observed in the AI. Additionally, moderately abundant such as *Stenotrophomonas* and *Actinomyces,* increased, while *Knoellia* decreased. Low-abundance genera such as *Blastopirellula, Persicirhabdus,* and *Pir4 lineage* increased in fish fed the PM diet, whereas *Caulobacter* and *IMCC26207* were higher in fish fed the CTRL diet.

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Dietary supplementation with the PM extract did not affect growth performance or overall microbial diversity, consistent with previous findings in Nile tilapia [6], where the main effects were observed in the increase of immune responses against bacterial infection by *Aeromonas hydrophila*. However, differential abundance analysis revealed slight, microbiota region-specific modulations, particularly in the PI. The archaeal phylum Halobacteriota, represented by *Halococcus*, was significantly increased in both intestinal regions of fish fed the PM diet. Although typically associated with hypersaline environments, *Halococcus* has been reported in the intestine of other fish [7, 8]a rapidly expanding sector, meets the global surging demand for aquatic food. Pangasius, a highly valued freshwater species, has seen a significant increase in demand due to its adaptability and potential for high yields, making it a promising candidate for aquaculture in India. This study investigates the gut microbiome composition of Pangasius pangasius fish cultured in three different systems (biofloc, cage, and pond. Its enrichment may reflect selective pressure from polyphenols, which favors stress-tolerant taxa under oxidative or antimicrobial conditions [9]. Several moderately abundant genera were also affected by the PM diet. In the AI, *Planctomicrobium* increased; this genus, common in aquatic environments and other fish and shellfish species [10–12]genotoxic, and teratogenic damages. One consequence of this exposure is the dysbiosis of the gut microbiota, where the normal bacterial composition is modified. Herein, we assessed the effect of the exposure to water accommodated fraction (WAF, is involved in the degradation of complex organic matter [13]budding, non-pigmented and rosette-forming bacterium was isolated from a littoral wetland of a boreal lake located in Valaam Island, northern Russia, and designated strain P3T. Ellipsoidal to pear-shaped cells of this bacterium were covered with crateriform pits and possessed stalks suggesting a planctomycete morphotype. 16S rRNA gene sequence analysis confirmed that strain P3T was a member of the order Planctomycetales and belonged to a phylogenetic lineage defined by the genus Planctomyces, with 89 and 86 % sequence similarity to Planctomyces brasiliensis and Planctomyces maris, respectively. Strain P3T was a mildly acidophilic, mesophilic organism capable of growth at pH values between pH 4.2 and 7.1 (with an optimum at pH 6.0–6.5, pointing to a positive effect on the digestion of non-digestible compounds. In the PI, *Stenotrophomonas* and *Actinomyces* were more abundant in the PM-fed fish. *Stenotrophomonas,* reported as both pathogenic and commensal in fish, including in the core microbiota of European sea bass [14]and strongly modulated by host diet. In aquaculture, a current challenge is to feed carnivorous fish with plant-feedstuffs in substitution of fish meal, an unsustainable commodity. Plants have a limited nutritive value due to the presence of non-starch polysaccharides (NSP, produces extracellular enzymes relevant to digestion [15]; while *Actinomyces* is known for cellulose degradation and has been detected in fish intestine [16], supporting its potential digestive role. *Knoellia*, which decreased in response to the PM diet, has been linked to lipid and glucose metabolism via acetate production [17], and may indicate improved lipid utilization. In summary, dietary PM induced minor but functionally microbial shifts, enhancing the genera associated with digestion without compromising growth performance, which may have a potentially beneficial effect in medium- to long-term supplementation.

**Conclusions**

Dietary supplementation with PM extract did not affect growth performance or microbial diversity in juvenile European sea bass but induced subtle region-specific shifts in the intestinal microbiota. These changes included a higher abundance of Halobacterota and certain genera associated with digestive functions, such as *Planctomicrobium*, *Stenotrophomonas*, and *Actinomyces*. These results suggest a potential role for PM extract in modulating intestinal functionality. However, further studies are being conducted to explore its effects on immune response and disease resistance.

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# BACTERIAL PATHOGENS AND MICROBIOME STRUCTURE IN *Arca noae* LINKED TO MORTALITY EVENTS IN THE EBRO RIVER DELTA

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## Introduction

Noah's ark (*Arca noae*) is a commercially valuable marine bivalve distributed throughout the eastern Atlantic and the Mediterranean Sea [1]. Although not yet established as an aquaculture species, interest in its cultivation is increasing, as reflected by a growing number of publications [2–4]. Recently, mortality events have been reported in natural beds of A. noae, including areas adjacent to oyster and mussel farms, raising concerns about ecological impacts and potential risks to nearby shellfish production; however, a previous study did not identify causal agents [5]. Accordingly, we aimed to identify putative pathogens associated with A. noae mortality in the Ebro River Delta by integrating culture-based isolation and challenge assays with 16S rRNA microbiota profiling; to this end, organisms collected during a mortality event were compared with an adjacent non-mortality population.

## Materials and methods

Fifty-five specimens were collected in July 2022 from two areas near the Ebro Delta: Les Cases d'Alcanar, during a mortality episode (6M sampling), and L'Ampolla, where no mortality was observed (6N sampling). Samples from live and recently dead arks were cultured on marine agar, tryptic soy agar (TSA), and thiosulfate–citrate–bile salts–sucrose (TCBS) agar to isolate putative pathogens. Isolates were typed by ERIC-PCR fingerprinting, the 16S rRNA gene was sequenced, and pathogenicity was assessed in adult *Arca noae* under laboratory conditions (bacterial doses: $1.6x10^7$-$4.4x10^8$ cfu/ark). Additional samples were stored at −80 °C for microbiota analyses. DNA was extracted using the DNeasy® PowerSoil® Pro Kit, and the V3–V4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq (2 × 300 bp). Reads were processed and taxonomy assigned with QIIME 2 and DADA2 using SILVA v138.1; downstream analyses were performed in R (microbiome package). Statistical analyses included t-tests for alpha diversity and PERMANOVA for beta diversity, using mortality status as the grouping factor ($p \leq 0.05$). For putative causal pathogenic genera, we generated a PCA contribution biplot based on relative abundances, ran LEfSe, and built Neighbor-Joining phylogenies aligning pathogenic isolates with microbiota ASVs.

## Results and discussion

***Isolation of potential pathogenic strains*:** From samples 6M and 6N, 166 isolates were obtained. ERIC-PCR typing of these isolates revealed two groups of isolates that were present only in the 6M samples. Based on 16S sequencing, these two groups were provisionally identified as *Acinetobacte*r sp. (*johnsonii)* and *Enterobacter* sp. Both groups were detected together in 83% of the 6M samples, comprising 14% and 12% of the isolates obtained from these samples. Virulence assays performed on representative isolates from these two groups (*Acinetobacter* sp. r499 and *Enterobacter* sp. r488) showed mortality rates of 80–90% and 80–83%, respectively. However, the control groups also showed high mortality (23–30%) over the 4 days of the assays.

***Microbiota profiling:*** Across 22 samples (each sample consists of a pool of 5 individuals), 1,019,430 clean reads yielded 7,326 ASVs; 766 ASVs were shared between mortality and non-mortality, accounting for 89.2% of total relative abundance (RA). Alpha diversity was lower in mortality than in non-mortality ($p \leq 0.05$): Observed richness 456 ± 107 vs 767 ± 2.21 ASVs; Shannon 2.56 ± 0.29 vs 3.85 ± 0.72; Faith's PD 25.75 ± 4.65 vs 38.56 ± 7.49. Beta diversity also differed significantly between groups ($p \leq 0.05$), indicating marked compositional dissimilarity.

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***Community composition:*** Mortality samples were dominated (relative abundance, RA > 5%) by Cyanobacteria (48%) and Proteobacteria (37%), whereas non-mortality samples were dominated by Cyanobacteria (31%), Spirochaetota (25%), Proteobacteria (16%), Firmicutes (9%), and Crenarchaeota (8%). At the genus level, mortality samples were dominated (RA > 3%) by *Synechococcus* CC9902 (45%), *Stenotrophomonas* (21%), *Acinetobacter* (4%), and *Spirochaeta* 2 (4%); *Enterobacter* was less abundant (1%). In contrast, non-mortality samples were dominated by *Synechococcus* CC9902 (25%), *Spirochaeta* 2 (25%), *Candidatus Nitrosopumilus* (8%), *Mycoplasma* (8%), and *Cyanobium* PCC-6307 (5%), with *Acinetobacter* (0.2%) and *Enterobacter* (0.01%) at very low abundance.

***Integration of culture and sequencing evidence:*** PCA (phylum/genus; Dim1 = 42.3%/45%) separated samples by mortality status: mortality aligned with potentially opportunistic taxa, Cyanobacteria (*Synechococcus* CC9902) and Proteobacteria (*Acinetobacter*, *Stenotrophomonas*, *Enterobacter*), whereas non-mortality associated with Bacteroidota, Firmicutes, Actinobacteriota, Verrucomicrobiota, and genera such as Sva0996 marine group, *Mycoplasma*, *Ruegeria*, *Blastocatella*, and *Cyanobium* PCC-6307. LEfSe (LDA > 3.5; $p \leq 0.05$) corroborated these patterns, identifying *Stenotrophomonas*, *Synechococcus* CC9902, *Acinetobacter*, and *Enterobacter* as mortality markers. Neighbor-Joining phylogenies were used to link pathogenic isolates to community ASVs; the ASVs most closely related to the isolates were more abundant in mortality (six *Acinetobacter*: ASV_15, ASV_30, ASV_414, ASV_77, ASV_122, ASV_21; two *Enterobacter*: ASV_78, ASV_29), consistent with genus-level relative abundances (*Acinetobacter* 4.1% vs 0.18%; *Enterobacter* 1.0% vs 0.01%).

Overall, in line with the PCA, LEfSe, and phylogenetic tree results, Acinetobacter and Enterobacter were consistently linked to the mortality event, and both have been reported in mollusk disease contexts [6, 7]. Stenotrophomonas and Synechococcus CC9902 also tracked the mortality state but were not recovered by culture, underscoring a limitation of culture-dependent approaches; Stenotrophomonas is typically an opportunistic, multidrug-resistant taxon in aquaculture microbiomes rather than a confirmed bivalve pathogen [9–11], whereas Synechococcus CC9902 is a ubiquitous coastal picocyanobacterium with no established pathogenicity in bivalves [12, 13].

## Conclusions

Mortality in *A. noae* was associated with marked microbiome shifts, lower alpha diversity and enrichment of Proteobacteria, with clear group separation by ordination and LEfSe. Challenge assays and phylogenetic mapping implicated *Acinetobacter* and *Enterobacter*. By contrast, *Stenotrophomonas* and *Synechococcus* CC9902 tracked mortality in sequencing data but were not recovered by culture; given reports of *Stenotrophomonas* as an opportunistic (occasionally pathogenic) taxon in aquaculture microbiomes, it remains a provisional candidate warranting targeted isolation and virulence testing. Collectively, these findings highlight *Acinetobacter* and *Enterobacter* as priority targets for surveillance and mechanistic studies in future mortality events.

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# FISH MATTER: A DIGITAL ECOSYSTEM TO UNLOCK THE VALUE OF AQUACULTURE BY-PRODUCTS

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## Introduction

Aquaculture and fisheries generate a large volume of by-products that are often discarded or underutilized, despite their high potential for valorisation in areas such as nutraceuticals, cosmetics, bioplastics, and animal feed. According to FAO (2022), improving the use of fish side-streams is a key pathway for increasing resource efficiency and sustainability in blue food systems. In the context of a circular bioeconomy, the effective valorisation of marine by-products is both an environmental and economic imperative (European Commission, 2020). Responding to this challenge, Fish Matter was developed as a digital platform to promote the sustainable and innovative use of marine by-products. Financed by Next Generation EU under the Blue Bioeconomy Pact, it aims to connect value chain actors and foster a knowledge-based innovation ecosystem. More information is available at https://fishmatter.pt.

## Objectives

Fish Matter aims to:

1. Serve as a unique virtual space connecting companies that generate marine by-products with those that transform them, along with technology providers.
2. Facilitate business matchmaking while promoting transparency and traceability.
3. Host a wider innovation ecosystem, integrating scientific networks, legislative frameworks, intellectual property resources, and funding opportunities.
4. Support the development of new projects and ideas, creating a dynamic knowledge base for collective intelligence and innovation.

## Material and methods

The platform was co-designed with actors from across the aquaculture and fisheries value chains, integrating feedback from industry, R&D institutions, and public agencies. It includes tools for registering available by-products, sharing technical and market data, and exploring potential applications across sectors such as nutraceuticals, cosmetics, animal feed, and biomaterials. A participatory approach involving surveys, pilot case studies, and stakeholder workshops guided the iterative development between 2023 and 2025.

## Results

Initial deployment in Portugal mapped a wide array of underutilized by-products and highlighted the need for digital tools to enhance connectivity, knowledge sharing, and trust. Early adopters will benefit from increased market visibility, and collaborations will be initiated through the platform. Stakeholders already registered valued the integrated access to scientific resources and regulatory guidance, recognizing Fish Matter not only as a marketplace, but as a catalyst for systemic innovation. With its integrated AI-powered chatbot, Fish Matter allows users to access tailored guidance, navigate legislation, identify funding opportunities, and explore suitable partners or technologies, significantly improving user experience and decision-making.

## Conclusions

Fish Matter exemplifies how digital innovation can drive circularity and value creation in aquaculture. Beyond matchmaking, it nurtures a collaborative environment for developing solutions, sharing knowledge, and scaling best practices. As interest in sustainable marine resources rises globally, Fish Matter positions Portugal as a pioneer in transforming marine co-products into engines of economic, environmental, and scientific impact, with potential for international replication.

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# RAINBOW TROUT *Oncorhynchus mykiss* CHRONIC STRESS RESPONSES INDUCED BY HIGH REARING TEMPERATURES: A HOLISTIC APROACH

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## Introduction

Increasing water temperatures arising from heat waves and global warming can lead to stressful conditions for farmed fish. Fish have developed mechanisms to adapt to challenging external environments and the ability to cope with stress, is a major health trait that can determine fish susceptibility to opportunistic pathogens and disease. However, rapid temperature changes can exceed the organism capacity to adapt to new homeostatic states. Stress resilience and fish welfare, within a context of changing environmental conditions, is rapidly becoming a major research subject in aquaculture. The present work aimed to assess the effects of high temperature-induced chronic stress on stress resilience as well as immune and oxidative stress responses of rainbow trout (*Oncorhynchus mykiss*) juveniles.

## Material & Methods

A trial with rainbow trout juveniles was devised. Three different groups were created, namely: CTR (16 °C); MED (19 °C); HIGH (22 °C). In each group, fish were randomly distributed into 6 tanks with 20 fish each (approx. 20 g initial body weight). Following an acclimatization period, fish were hand fed a fixed amount of feed (3% body weight) 2 times a day with a commercial-like diet, for 4 weeks. After this period, blood samples were collected for hematological procedures while plasma was isolated and used for cortisol, metabolites and humoral immune parameters. Liver was sampled for the analysis of metabolites and oxidative stress responses. Head-kidney, brain and hypophysis were sampled for gene expression analysis. Skin mucus and water were sampled both for proteomics and transcriptomics (miRNA) analyses, while skin tissue was collected solely for RNAseq analysis.

## Results and Discussion

Results from the analysed plasma humoral parameters showed no differences between experimental groups regarding anti-proteases activity, after 4 weeks. However, the HIGH temperature group showed higher lysozyme activity than CTR. Regarding the head-kidney panel of selected genes, glucorticoid receptor 1 (gr1) and arginase 2 (arg2) showed significantly higher gene expression in HIGH temperature group in comparison to MED, although no differences were perceived relative to CTR.

Chronic stress is associated with glucocorticoid receptor loss of sensitivity, impairing corticosteroids regulatory effects, namely the anti-inflammatory function. It has also been linked with increased circulating pro-inflammatory biomarkers in blood. Ongoing data analysis on the above referred tissues and matrices will allow a better understanding of stress and health related mechanisms involved in chronic stress response, namely the relation between the HPI axis and the immune mechanisms. The integration of all the generated data, is expected to provide the establishment of reliable biomarkers under chronic stress conditions.

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# IMPROVING GROWTH AND FEEDING BEHAVIOURS OF FARMED SHRIMP *Penaeus vannamei* IN COMMERCIAL CONDITIONS

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## Introduction

Over 400 billion shrimps are farmed each year worldwide, more than five times the total number of all farmed land animals. Despite these facts, our scientific knowledge on shrimp behaviour and welfare is quite weak.

This study focuses on the feeding behaviour of *Penaeus vannamei*. According to the literature, shrimp feed in small quantities; offering feed increases exploration, crawling and feed grabbing; while feed-deprivation and molt status influence the feeding behaviour. Nevertheless, there are controversial studies regarding the preferred feeding time by shrimps and the best feeding frequency. Moreover, the way shrimp behave in groups during feed distribution is still unknown.

## Material and methods

The study aimed to observe shrimp behaviour directly in raceways in commercial farms, with one raceway being fed every hour during daytime (from 7 am to 7 pm) and the other being fed every hour during day and night, with the same feed quantity per 24h in both raceways. The behaviour of shrimp has been recorded every three days, four times a day, for 15 minutes around feeding time via GoPro. These videos have been analyzed based on an ethogram designed for this study. Water quality has been recorded several times a day and 60 shrimp have been sampled every day to score eight health indicators to see how shrimp react to different feeding schedules. To estimate shrimp activity levels, we performed image scans every 30 seconds over the course of 15-minute video recordings. At each scan (i.e., video still frame), we counted the number of shrimps that were resting on the substrate versus those that were suspended in the water column. This snapshot-based approach allowed us to quantify temporal patterns of activity based on spatial positioning. Furthermore, weight has been recorded every two days based on a shrimp sample leading to a weight mean.

## Results

One of the main concerns of farmers was the water quality and the possible deterioration in case of frequent feeding. The results showed no difference in the two raceways which is good news for producers. Furthermore, concerning weight, shrimp fed day and night gain more weight than those fed only during the day.

The scan sampling based on videos revealed that shrimp fed only during the day were significantly more active—i.e., more frequently observed in suspension—during the feeding compared to shrimps fed day and night. This higher activity for shrimps fed during the day only was also observed before the first feeding of the day (7 am) suggesting an anticipatory response to food availability.. To conclude, feeding the shrimp every hour during night and day lead to have behaviours closer to natural shrimp behaviours which tend to rest more on the substrate.

## Conclusion

It is a pioneer study as it has never been done to observe and analyze shrimp behaviour in commercial farms without using AI. A better understanding of shrimp feeding behaviour will help many farmers and also improve the lives of many shrimps in commercial farms.

More similar studies would be a key to confirm the different results, especially concerning the weight to ensure that all shrimps gain more weight.

# SAMPLING SHRIMP (*Penaeus vannamei*) THE RIGHT WAY: A BETTER ANTICIPATION OF HEALTH ISSUES

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## Introduction

Over 400 billion shrimps are farmed each year worldwide, more than five times the total number of all farmed land animals. Despite these facts, our scientific knowledge on shrimp behaviour and welfare is quite weak.

Animal monitoring is typically conducted daily in terrestrial farms to assess the health of individuals and identify potential issues. In shrimp ponds, however, the main challenge lies in the brackish water, which hampers visibility and makes it difficult to observe the animals' behaviour. In many cases, farmers sow their pond with post-larvae and then sample a variable number of shrimps at different times during the grow-out stage. In ponds with automatic feeders, it is common for farmers to realize these samples around the feeders to catch enough shrimp to monitor. However, the risk is not only to have the biggest and healthiest shrimp but also to miss some information on the health status of the pond. Furthermore, a low number of indicators are usually recorded. According to the literature, more indicators can be used to have a clearer view of shrimp health with some indicators being linked indirectly to welfare (Pedrazzani et al. 2023). This study aimed to determine (i) where to sample shrimp in the pond, and (ii) how many and (iii) which indicators are useful to better detect health issues.

## Material and methods

To answer these questions, 350 shrimps (*Litopenaeus vannamei*) have been sampled with 50 per sampling point distributed at seven different areas of the pond, twice a week, at two different times (morning and afternoon) and in two commercial ponds. After 1 to 3 net throws in an area to obtain at least 50 shrimps, the farmer could move away and collect shrimp in a different location to avoid spending hours at the same spot. It leads to a total of 2,550 shrimp being sampled during the duration of the trial for one pond and 4,865 for the second pond. Eight health indicators have been scored (rostrum, eyes, antennae, gills, hepatopancreas, exoskeleton, uropods, musculature, motor appendages) based on the study from Pedrazzani (2023) with score 1 (good welfare), 2 (tolerable welfare) or 3 (critical welfare). The shrimp were sampled at the inlet, at the outlet, around 2 automatic feeders and close to 3 edges.

Moreover, oxygen, temperature and salinity were recorded once or twice a day in addition to weekly weight estimation, percentage of survival, biomass, quantity of feed distributed and usual notation of percentage of shrimp in good health. Scores were analyzed using linear models and non-linear models (generalized additive model) to evaluate the health variation of shrimps over the days in both ponds.

## Results

The results first show that the localization for global health monitoring is important (significant variations in health scores depending on the localization). In both ponds, the health deteriorates faster over the days in the grow-out pond at the edges and at the outlet of the pond. Some health indicators vary over time with certain indicators being more efficient than others to detect special events. For example, motor appendages seem to be a good indicator as we observed scores stabilizing after a potential weather event and a reliable indicator as score variation is largely explained by days, for both ponds.

This stabilization could be explained by a high mortality after the weather event, leading to have only the shrimp in better health staying in the pond. After some time, there is once again a deterioration of the health.

## Conclusion

This protocol not only encourages farmers to focus more on shrimp welfare but also allows to determine where samplings are more useful to better anticipate issues and thus, improve shrimp health and welfare by reacting faster. Implementing this monitoring would also contribute to increase productivity.

# METATRANSCRIPTOMIC TOOLS FOR THE DETECTION OF PATHOGENS IN MOLLUSCS

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## Introduction

Metatranscriptomics has emerged as a very useful technology for the study of microbiomes from RNA-seq reads. This method provides additional information compared to the sequencing of ribosomal genes such as the possible detection of viral sequences or the gene expression evaluation of both, host and associated microorganisms (dual-transcriptomics). Moreover, the targeted search for specific and active pathogens is a valuable strength.

Molluscs industry, and especially bivalves' market is an economic sector of high potential in the Spanish coast, especially in Galicia. These species cope with highly variable environmental conditions in estuarine and intertidal regions. However, the current climate change scenario implies severe changes such as temperature increase, acidification, variations in water salinity or oxygen availability. This have a direct effect on the marine ecosystem and therefore on the species that are cultivated, which are fully adapted to specific conditions. Furthermore, there emerge additional risks such as the appearance of new pathogens, which may result in production problems.

In addition to the risk posed by the emergence of new diseases, there are a significant number of known bivalve pathogens that are already causing problems for cultures today. For instance, clams are susceptible to bacterial diseases (mainly vibriosis caused by *V. splendidus*, *V. alginolyticus* or *Vibrio tapetis*) and also multiple protozoan parasites such as *Bonamia*, *Perkinsus*, *Haplosporidium* or *Marteilia*, which invade tissues and have a negative impact on reproduction and growth. Another notable case is the high susceptibility of oysters to the OsHV-1 herpesvirus. Mollusc diseases have a common denominator, which is the absence of treatment, therefore the only possibility of control lies in prevention and monitoring of stocks.

## Material and methods

Datasets available in public databases were used to perform a meta-analysis. Prior to the analysis, raw reads (RNA-seq reads) were trimmed to remove sequencing adapters and short and low-quality sequences (quality score limit 0.01 = PHRED 20). Moreover, datasets were randomly subsampled to equal the lowest number of reads to compare microbial abundance in a more appropriate manner. To analyze the different microbiome profiles, a working plan consisting of mapping the reads to several reference databases was implemented. The microorganism reference databases were built with all the virus, protozoan, and fungal genomes deposited in RefSeq. Specifically, these databases contained a total of 2,396 bacterial sequences, 10,907 viral sequences, 93 protozoan assemblies, and 388 fungal assemblies. Available genome assemblies were used to filter putative host-specific reads. Mapping parameters used to classify read packages in different taxonomic groups were as follows: length fraction = 0.5, similarity fraction = 0.8, and minimum seed length = 50. This minimum seed length parameter defines the minimum perfect match length for a position in the reference to be considered a valid candidate when matching the read.

## Results

In this work, we used the metatranscriptomic approach to study a series of relevant mollusc pathogens in available transcriptomic data, to have a broad view of the prevalence of several pathogens in different geographical locations.

Moreover, we could also detect other associated microorganisms, being possible to stablish some relationships and interactions.

# EVALUATION OF ANTI-FOULING COATINGS AGAINST GOLDEN MUSSEL ON NETTING FOR CAGE AQUACULTURE

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**Introduction**

Biofouling caused by the golden mussel (*Limnoperna fortunei*) compromises the integrity of cage aquaculture structures, increasing operational costs and sanitary risks. This study evaluated two types of antifouling coatings as alternatives to conventional biocides: a commercial hybrid resin of silicone and epoxy, and a biocidal paint containing lysoglycerophosphocholines (LGPCs), derived from soybean lecithins.

**Material and methods**

In September 2024, 36 metal frames (80 × 130 cm) were installed in the Chavantes Reservoir, Paranapanema River, at the Southeast–South border of Brazil. The frames were distributed across three sectors of a fish farming. Each frame, submerged at a depth of 0.5 m, contained three stainless steel mesh panels (20 × 20 cm): one uncoated (control), one coated with a hybrid silicone-epoxy resin, and one coated with biocidal paint.

From September 2024 to April 2025, monthly 1000 L water samples were collected, filtered (40 µm), and preserved for quantifying *Limnoperna fortunei* larvae. Each month, one mesh panel was randomly selected; individuals in a central 10 × 10 cm area were collected and counted. Larvae and juveniles were counted by stereomicroscopy (Sedgwick-Rafter method), and adults (>5 mm) by visual inspection. A two-way ANOVA assessed differences in total attached larvae and adult densities across sectors and materials.

The coatings were subjected to physical tests for blister formation and immersion in a specialized laboratory, as well as ecotoxicological tests, exposing *Daphnia similis* and *Ceriodaphnia dubia* to stainless steel wires coated with the tested materials and submerged in 10 mL of synthetic medium (SM).

**Results and Discussion**

*L. fortunei* larvae were detected throughout the study in the environment, with density peaks in November, February, and notably April, reflecting increased reproduction in warmer months. This was supported by higher larval and juvenile adhesion from December and increased adult presence from January (Table 1). The highest adult fouling on the mesh panels with the three materials was observed in Sector 3, in the month of April. (Figure 1).

The coatings did not reduce *L. fortunei* fouling compared to uncoated mesh ($F=0.13$; $p=0.87$), but differences were observed between sectors ($F=12.38$; $p=0.00$). Tukey's post hoc test showed that sector 1 differed from sectors 2 and 3. In sector 1, panels were covered by filamentous algae (Oscillatoriaceae), which may have hindered mussel attachment.

In the blistering test, paint peeling began after 880 hours. In the immersion test, gloss and color changes appeared after 240 hours, and resin peeling started after 760 hours. Ecotoxicological assays revealed acute toxicity of the paint to *D. similis* and chronic effects on *C. dubia*, while the resin caused chronic effects on *C. dubia*. These findings indicate coating degradation and/or toxic substance release, potentially compromising antifouling efficacy and posing environmental risks. Improving the durability and performance of these materials is recommended for future assessments.

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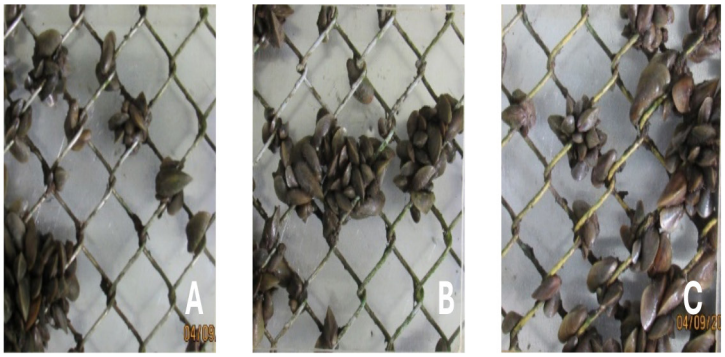


Figure 1. *Limnoperna fortunei* on stainless steel mesh panels (April, Sector 3): A) Uncoated; B) Hybrid silicone-epoxy resin; C) Biocidal paint.

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Table 1. Density of *L. fortunei* larvae in the environment and adhered individuals on different panel materials in a fish farming area. SS = Stainless steel

| | | **Environmental larvae (ind/m³)** | **Adhered larvae /juveniles (ind/m²)** | | | **Adhered mussels (ind/m²)** | | |
|---|---|---|---|---|---|---|---|---|
| Date | Sector | Mean | SS | Resin | Paint | SS | Resin | Paint |
| 10/10/2024 | 1 | 372 | 100 | 0 | 0 | 0 | 0 | 0 |
| 10/10/2024 | 2 | 259 | 0 | 0 | 100 | 0 | 0 | 0 |
| 10/10/2024 | 3 | 1521 | 0 | 0 | 300 | 0 | 0 | 0 |
| 13/11/2024 | 1 | 2811 | 100 | 100 | 200 | 0 | 0 | 0 |
| 13/11/2024 | 2 | 2069 | 300 | 300 | 400 | 0 | 0 | 0 |
| 13/11/2024 | 3 | 1757 | 300 | 700 | 0 | 0 | 0 | 0 |
| 10/12/2024 | 1 | 1004 | 100 | 200 | 200 | 0 | 0 | 0 |
| 10/12/2024 | 2 | 800 | 1000 | 1000 | 1500 | 200 | 0 | 0 |
| 10/12/2024 | 3 | 866 | 2500 | 2200 | 3600 | 0 | 0 | 0 |
| 08/01/2025 | 1 | 779 | 100 | 200 | 100 | 0 | 0 | 0 |
| 08/01/2025 | 2 | 1163 | 1100 | 1200 | 600 | 1000 | 500 | 1200 |
| 08/01/2025 | 3 | 800 | 9800 | 8900 | 3000 | 3800 | 1900 | 2100 |
| 10/02/2025 | 1 | 5311 | 100 | 0 | 600 | 0 | 0 | 200 |
| 10/02/2025 | 2 | 4960 | 5400 | 3500 | 2900 | 3500 | 1700 | 4100 |
| 10/02/2025 | 3 | 3821 | 5100 | 2700 | 7400 | 7300 | 6800 | 15800 |
| 13/03/2025 | 1 | 1352 | 900 | 1300 | 0 | 100 | 100 | 100 |
| 13/03/2025 | 2 | 1446 | 2900 | 2200 | 2500 | 4000 | 6800 | 5900 |
| 13/03/2025 | 3 | 2275 | 3500 | 2100 | 1500 | 22800 | 9200 | 17000 |
| 09/04/2025 | 1 | 15908 | 500 | 1000 | 300 | 300 | 100 | 400 |
| 09/04/2025 | 2 | 14951 | 2300 | 4700 | 2700 | 20100 | 7300 | 9700 |
| 09/04/2025 | 3 | 11757 | 2200 | 900 | 3600 | 11800 | 12700 | 11300 |

# EFFECTS OF PARTICLE POLLUTANTS PRODUCED THROUGH IN-WATER HULL-CLEANING PROCESS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF WHITELEG SHRIMP, *Litopenaeus vannamei*

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We conducted a comprehensive assessment involving acute effects on 96-hour survival and biochemical parameters of the whiteleg shrimp, Litopenaeus vannamei exposed to different concentrations of particle pollutants produced through in-water hull-cleaning process to evaluate the potential impact of ship hull-cleaning wastewater on crustaceans. The analyzed particle pollutants contained high concentrations of metals and antifoulants. The results revealed dose-dependent reductions in survival rates, accompanied by a notable increase in oxidative stress and reduction in feeding rates. Molecular and biochemical responses were then analyzed at the transcriptomic, proteomic, and metabolomic levels, after integration with in silico bioinformatics. Our findings revealed that the primary toxic mechanism of particle pollutants involved the induction of oxidative stress and detoxification pathways, reduction in immune pathways, and increase endoplasmic reticulum stress, leading to cell death. Collectively, these findings underscore the potential harm posed by particle pollutants to the health of crustacean populations under aquaculture systems.

# EXPLORING SEX-DRIVEN EPIGENOMIC RESPONSES TO INFECTIONS

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## Introduction

Bacterial infections are a major cause of disease outbreaks in fish, leading to reduced productivity in aquaculture. Understanding the molecular responses triggered in the gonads following infection is crucial for improving fish farming practices, optimizing disease control, and identifying the most resilient phenotypes (Caballero-Huertas et al., 2024). This study focuses on two key species: the commercially valuable European sea bass (*Dicentrarchus labrax*), widely farmed across Europe, and the extensively studied model organism zebrafish (*Danio rerio*). We investigated epigenomic alterations in both species following bacterial challenge. The epigenomic approach consisted on state-of the arts techniques such as Whole Genome Methylation sequencing (WGMS), microRNA-seq, and RNA-seq.

## Methodology

European sea bass and zebrafish were experimentally exposed to bacterial pathogens. In European sea bass, infections were administered via intraperitoneal injection, and gonadal tissues were collected 48 hours post-infection. For zebrafish, exposure occurred through bath immersion. Sequencing libraries were constructed according to standard manufacturer protocols for each specific sequencing technique. Additionally, the expression of selected key microRNAs (miRNAs) was analyzed using the miRCURY LNA SYBR Green PCR Kit.

## Results

Bacterial challenge led to substantial alterations in the gonadal epigenome, transcriptome, and miRNome. The most pronounced changes in DNA methylation were observed between male and female samples under the same treatment, with over 88,000 and 105,000 hypermethylated CpG sites, and approximately 3 million and 2.7 million hypomethylated CpG sites, respectively. Transcriptome profiling of European sea bass gonads indicated a higher number of differentially expressed genes (DEGs) in testes compared to ovaries. Four miRNAs were identified in testicular tissue as potential biomarkers of prior infection exposure. In zebrafish, analysis of gonadal miRNA expression revealed sex-dependent differences post-infection, including suppression of reproductive pathways such as oocyte meiosis, and activation of immune-related pathways.

## Discussion

As similarly reported by Gelderen et al. (2025a, 2025b), the sex-specific epigenetic differences observed may underlie the sexually dimorphic responses to infection. These results highlight the significance of incorporating sex as a biological variable in fish immunological and epigenetic research. Although sexual dimorphism is known to modulate immune function across vertebrates, its role in fish immunity remains barely underexplored (Caballero-Huertas et al., 2024). This study enhances our understanding of the sex-driven epigenomic basis in fish, with important implications for aquaculture practices and the development of disease-resistant phenotypes.

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# VITAMIN D METABOLISM AND THE IMPLICATIONS OF FEED WITHDRAWAL

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## Introduction

Vitamin D is essential for salmonids, yet its metabolism and physiological roles in these species remain incompletely understood. To exert its biological effects following absorption, dietary cholecalciferol ($D_3$) must be metabolized *in vivo* to calcifediol (25-OH-$D_3$), and subsequently to its active form, calcitriol (1,25-OH-$D_3$). Supplementing with 25-OH-$D_3$ can be effectively improve vitamin D status in fish by partially bypassing this metabolic process (Rider et al., 2024 & 2023). This study investigated the effects of oral intake of these plasma metabolites on vitamin D metabolism and calcium (Ca) and phosphorus (P) homeostasis by analysing their kinetics.

## Methods

Single 22 $\mu$g oral doses of $D_3$ or 25-OH-$D_3$, or a 2.54 $\mu$g dose of calcitriol-glycosides (1,25-OH-$D_3$-gly) were administered to rainbow trout fed a practical diet containing 2,817 IU/kg $D_3$. The kinetics of circulating vitamin D metabolites, calcium (Ca), and phosphorus (P) were monitored in plasma over six days by sampling at 6, 24, 48, 72 and 168 hours post-gavage. A 42-hour feed withdrawal period was associated with the administration of the single oral doses.

## Results

In fish receiving the baseline diet and prior to the gavage, neither $D_3$ nor 25-OH-$D_3$ were detectable in plasma, while 1,25-OH-$D_3$ was the most abundant vitamin D metabolite. A single dose of $D_3$ produced a plasma $D_3$ peak at 48 hours, whereas a single dose of 25-OH-$D_3$ resulted in a smaller 25-OH-$D_3$ peak at 72 hours. The $D_3$ peak did not alter baseline 25-OH-$D_3$ levels. A single dose of 1,25-OH-$D_3$-gly did not influence baseline 1,25-OH-$D_3$ levels. None of the single vitamin D doses significantly altered plasma levels of active vitamin D, Ca, or P.

Across all groups, plasma 1,25-OH-$D_3$ was significantly modulated over time. Plasma 1,25-OH-$D_3$ levels significantly declined reaching a minimum 24 hours after gavage and were not restored by any of the vitamin D treatments, see Figure 1. Circulating Ca, and especially P, also declined 24-hours after the drop in plasma 1,25-OH-$D_3$.

## Conclusions

These findings demonstrated that even at 1.7 times the NRC minimum of 1,600 IU/kg $D_3$, neither $D_3$ nor 25-OH-$D_3$ were detectable in plasma. Although both $D_3$ and 25-OH-$D_3$ were rapidly absorbed, they were also rapidly cleared. The observed kinetics also indicated that short-term feed deprivation significantly reduced active vitamin D levels, and this decline was not mitigated by the single doses of any of the vitamin D sources. The subsequent reductions in plasma Ca and P suggest a temporal link with declining plasma 1,25-OH-$D_3$ levels. This is the first study to connect circulating active vitamin D to feed intake in fish, pointing to a potential role of this vitamin in energy metabolism.

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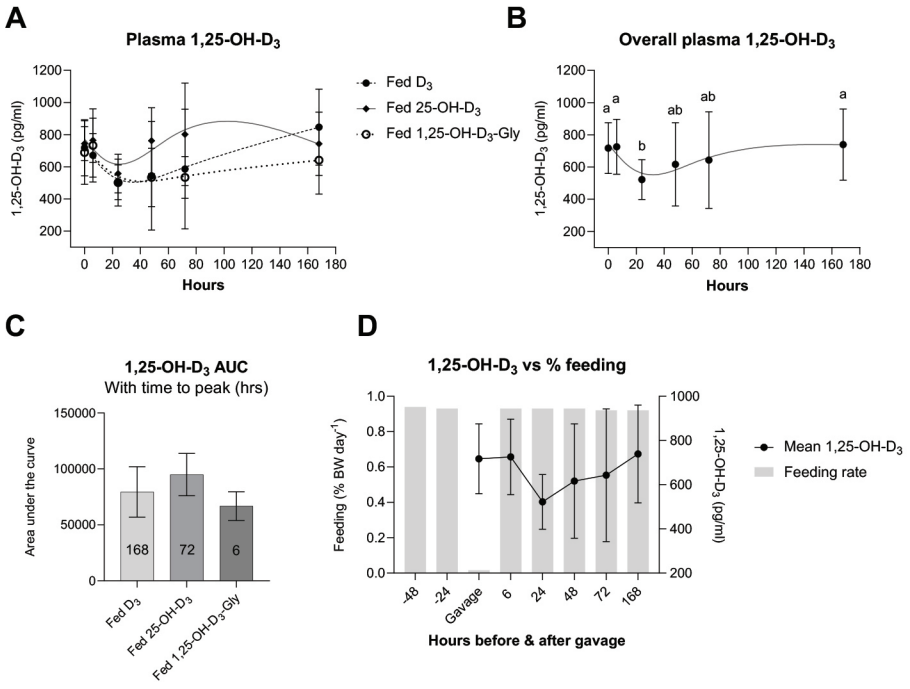


**Figure 1.** Plasma 1,25-OH-$D_3$ levels over 168 hours after administration of single doses from different vitamin D sources.

A: Repeated measures mixed model (nested) analysis; time effect, $p = 0.00002$; vitamin D source, $p = 0.27992$; time × source interaction, $p = 0.8881$.

B: Post-hoc Tukey HSD test, time points not connected by the same letter differ significantly.

C: One-way ANOVA for area under the curve (AUC), $p = 0.5874$.

D: Plasma 1,25-OH-$D_3$ levels overlaid with feeding rate.

# HEALTH BENEFITS OF A NOVEL PREBIOTIC OLIGOSACCHARIDE FOR AQUACULTURE PRODUCTION

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## Introduction

Growth in aquaculture production is contingent upon strategies to manage and mitigate infections that reduce growth and harvest quality and that lead to premature mortality. Aquatic diseases caused by pathogenic bacteria, viruses, and parasites are the most pressing issues affecting aquaculture producers and the most acute within intensive aquaculture production systems.

Prebiotics – compounds that selectively feed beneficial bacteria within the body - can play an important role in supporting digestive and immune health in aquaculture species. GreenSage Prebiotics uses residues from coconut production to produce a novel prebiotic, Pretego™, that supports digestive and immune health in aquatic and terrestrial animals. Pretego™ is unique and distinct from other prebiotics, owing to its ability to directly inhibit the growth of several bacteria that have a material impact on aquaculture production, confirmed by MIC studies (Table 1).

In this presentation, we report on the impact of Pretego™ on the health and survival of shrimp and finfish (e.g., Atlantic salmon and sea bass).

## Materials and Methods

***Trial 1***: A 42-day feeding trial was conducted at the ShrimpVet Demonstration Farm (Vietnam), followed by a pathogen challenge with *Vibrio parahaemolyticus*. At the beginning of the trial, 60 SPF shrimp (*L. vannamei*) with an average body weight of 2.41 ± 0.49 g/shrimp were stocked into 22 tanks. The feeding trial consisted of 4 groups: (i) Control group (No Pretego™); (ii) 0.30 wt% Pretego™; (iii) 0.50 wt% Pretego™; and (iv) 0.65 wt% Pretego™. Following the feeding trial, the pathogen challenge trial was conducted by immersion in *Vibrio parahaemolyticus* for 8 days. The control group was subdivided into two subgroups, to include shrimp that were and were not exposed to the pathogen. Shrimp growth and survival data were collected.

***Trial 2***: Atlantic salmon (*Salmo salar*) parr (ca. 11.68 ± 0.11g) were fed either a control diet or diets supplemented with one of three levels of Pretego™ (0.25, 0.35 or 0.5%). Diets were manufactured by Skretting using commercially relevant formulations that met the nutritional requirements of Atlantic salmon. Fish were reared in a freshwater RAS system for 70 days, with 3 replicated tanks per treatment group. Fish growth performance was determined at the conclusion of the trial, and fish skin and intestinal samples underwent histological assessment.

## Results

***Trial 1***: Increasing the inclusion rate of Pretego™ was correlated with improved growth performance and feed efficiency in *L. vannamei*. Inclusion of Pretego™ at 0.5% and 0.65% led to significantly better outcomes, with up to 26% more biomass harvested (correlated with higher survival rates), higher average daily weight gain and specific growth rate, and a 24% lower feed conversion ratio compared to the control group that did not consume Pretego™. Following exposure to *Vibrio parahaemolyticus*, survival was dramatically higher in shrimp that consumed Pretego™ – increasing from 22% to 70% (Figure 1).

| | |
|---|---|
| *Vibrio parahaemolyticus, anguillarum* | *Tenacibaculum maritimum, finnmarkense* |
| *Moritella viscosa* | *Piscirickettsia salmonis* |
| *Aeromonas salmonicida* | *Yersinia ruckeri* |

Table 1: Aquaculture Pathogens Inhibited by Pretego™

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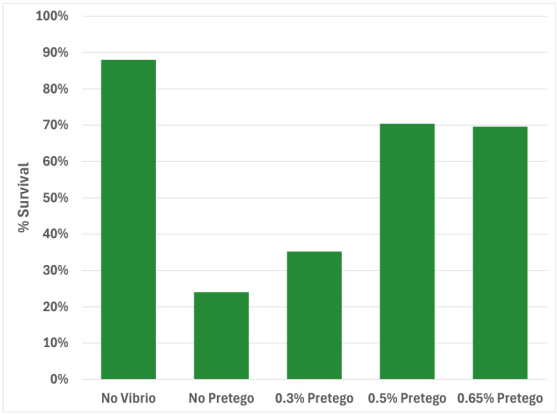


Figure 1: Impact of Pretego™ on Shrimp Survival Following an EMS Challenge

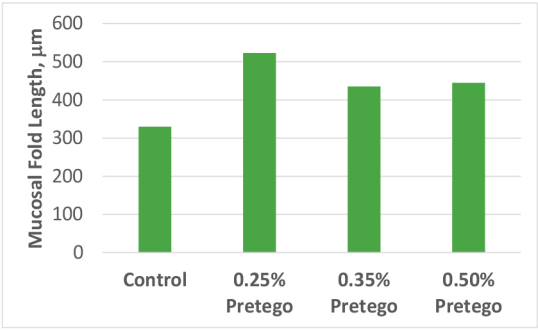


Figure 2: Impact of Pretego™ on Mucosal Fold Length

***Trial 2***: There were trends suggesting improvement in feed conversion and growth rate over the 70-day study, but these improvements were not statistically significant. Nonetheless, there were statistically significant increases in the mucosal fold length (an indicator of improved nutrient absorption; Figure 2), and the histological appraisal revealed that Pretego™ significantly increased goblet cell counts in the intestine and skin, which is indicative of improved disease barrier function. Samples from the trial mid-point revealed that benefits started to emerge from as early as day 35, with elevated mucosal fold length, intestinal goblet cell levels, and skin goblet cell levels compared to the control fish.

**Conclusions**

Pretego™, a novel prebiotic from coconut residue, supports the digestive and immune health of shrimp, salmon, and other aquaculture species. Pretego™ improves survival and growth when added to aquaculture feed at levels from 0.25% to 0.65%. Pretego™ supports aquaculture production during the grow-out phase and during periods when there is a high risk of infection.

# GENOME-WIDE MULTIPLEX AMPSEQ PROFILING OF *Neoparamoeba perurans*, MOLECULAR EPIDEMIOLOGY

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## Introduction

Amoebic gill disease (AGD) is a significant infection and health concern of farmed Atlantic salmon (*Salmo salar L.*), caused by the marine amphizoic amoeba *Neoparamoeba perurans*. Since its emergence in the mid-1980s, the disease has become a global challenge for aquaculture. Despite its impact, little is known about how *N. perurans* spreads between farms, production cycles, or across geographical regions – essential knowledge for effective control and fallowing strategies. Population-level genomic studies of *N. perurans* have been hindered by high levels of inter- and intracellular bacterial contamination that obscure parasite-derived sequence data. To date, only a few genetic markers have been sequenced, offering insufficient resolution to inform management practices and molecular epidemiological studies. To address this, a draft genome assembled by the Llewellyn lab enabled the development of 82 unique primer pairs targeting loci across nuclear, mitochondrial, and kinetoplastid genomes of *N. perurans*. These markers aim to address the gap by demonstrating that high-resolution genotyping can be achieved directly from field samples, supporting disease tracking and management.

## Materials and Methods

Gill swabs and gill tissue were analysed from 2019 to 2023, collected from multiple sites across Norway (n = 9), Scotland (n = 4) and Ireland (n = 1). Initially, a multiplex PCR panel comprising 201 individual primers was used to amplify loci across all targeted genomes (n =158). The resulting products were sequenced on an Illumina NGS platform following custom 3-round library preparation. Sequences from each sample were processed to remove orphan reads using fastq_pair, then mapped to the draft genome with BWA. Resulting SAM files were converted to BAM, sorted, and indexed using Samtools. BAM files were converted to BED format in Bedtools, sorted, and read counts per genomic contig were obtained using Linux cut and uniq commands. Mapping efficiency for each marker was then assessed in R using ggplot, dplyr, and tidyr.

Following initial sequencing, the primer pool was refined to 82 unique primer pairs targeting the genomes of *N. perurans* and kinetoplastid sequences. These markers were amplified individually via single-target PCR reactions, with products from each sample (n = 58) pooled and run on an agarose gel. Bands were gel-extracted using a custom protocol, then subjected to a custom library preparation involving 2$^{nd}$ and 3$^{rd}$ round PCRs to attach custom barcodes and sequencing adaptors. An ethanol precipitation step was performed after 3$^{rd}$ PCR, and the final products were pooled together and sequenced on an Illumina NGS platform. Bioinformatic analysis is ongoing.

## Results and Discussion

Following read mapping of the multiplex sequencing run, approximately 21.7 million reads were recovered across all samples (n = 158). Substantial dropout occurred across the amplicon panel, with many markers yielding zero reads, indicating reduced efficiency of amplification in a multiplex. After removing failed markers and duplicates, only 82 amplicons from the original panel were retained (Figure 1). Several biological samples were absent from the final dataset, suggesting inconsistencies in amplification. Read distribution was strongly biased: ~21 million mapped to kinetoplastid nuclear targets, none to the kinetoplastid mitochondrial, and only ~56,000 and 29 reads to *N. perurans* nuclear and mitochondrial genomes. Respectively. These results highlight an imbalance in primer performance and inefficient coverage across genomic targets. Therefore, the primers were reduced to 82 unique targets and run as single-target PCR reactions to improve coverage for unrepresented genomes of *N. perurans* and kinetoplastid mitochondrial targets. Analysis is currently ongoing to determine this approach's success.

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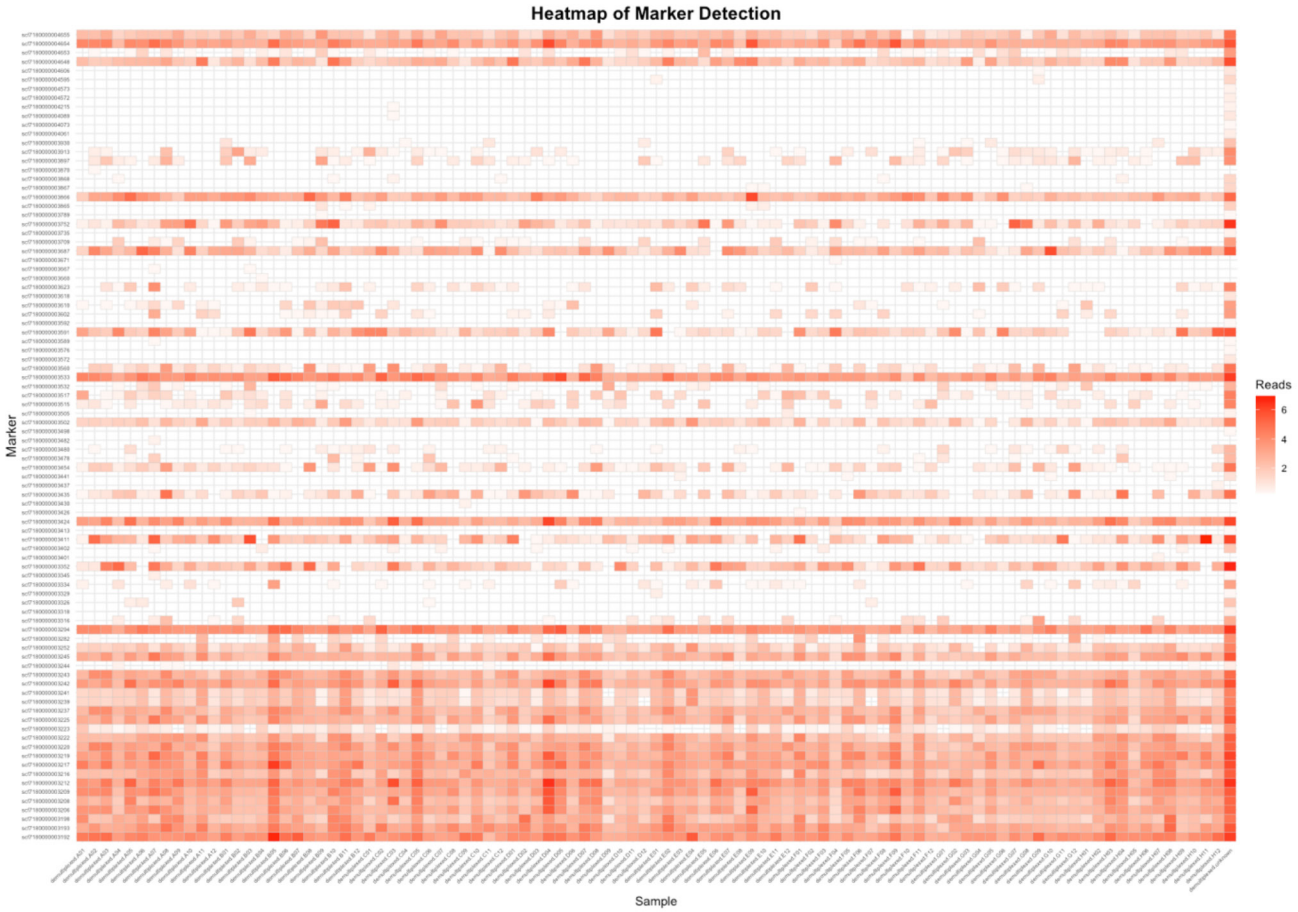


**Figure 1: Heatmap of amplicon marker detection across all biological samples.** Each cell represents the read count (log-transformed) for a given marker (y-axis) in a specific biological sample (x-axis).

## Conclusion and Future Work

To date, there is no reliable method to attribute AGD infections to a specific source, nor to effectively prevent parasite transmission between farms, production cycles, and the surrounding environment. This work takes a step toward addressing this gap by demonstrating that high-resolution genetic data can be derived directly from gill swab samples, providing valuable insights for the treatment and control of AGD. Future research will focus on delivering this information directly to farm managers through a web-based visualisation tool capable of tracking genotypes and their spread over time, enabling more targeted and timely responses to AGD outbreaks.

# BENCHMARKING OF HEALTH PROMOTING ADDITIVES TO SUPPORT HEAT STRESS TOLERANCE OF EUROPEAN SEABASS *Dicentrarchus labrax*

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## Introduction

Water temperature plays a crucial role in fish culture. In the context of climate change and extreme temperature events, the impact of increasing ocean temperature affects aquaculture production in general, and European aquaculture in particular. In the southern part of the Mediterranean, the temperature of shallow waterbodies may occasionally exceed 33–34 °C, especially during summer. European seabass, *Dicentrarchus labrax*, an important aquaculture species, is challenged with the increased frequency and intensity of heatwaves (Islam et al., 2025; Sáez-Arteaga et al., 2020) adding stress to farmed fish. Some of the strategies to mitigate thermal stress include feeding optimization, water quality control, culture density management and lately, dietary manipulations (Herrera et al., 2019). Feed additives have shown efficacy to counteract stress effects (including oxidative stress) and physiological functions in farmed fish (Zhang et al., 2020). In this study, we have done a benchmarking of different health additives to strengthen heat stress tolerance in European seabass.

## Material and methods

European seabass of 144 ± 3.23 g average individual body weight was distributed in 15 tanks of a marine RAS. A group of 14 animals was stocked per tank. Water temperature was controlled during the trial: 1st week at 26ºC; 9 weeks at 28ºC, 1 week at 32ºC and 10 days at 28ºC. Temperatures changes were applied progressively at 1ºC per day until the established temperature.

Five isonitrogenous and isolipidic diets (43 % CP/16% CF) were evaluated: 1) a control diet, 2)HeatPro (Adisseo), 3) Sanacore (Adisseo), 4) R2 and 5) K8. Fish were fed manually twice per day to apparent satiation 6 days per week. Feed intake was controlled daily. The trial lasted 12 weeks. After the 9 weeks at 28ºC seabass were sampled for SGR and FCR. At the end of the trial (12 weeks), fish were sampled to calculate SGR, FCR and somatic indexes. Plasma samples were also taken for cortisol, glucose, lactate, total proteins, TAG and cholesterol determinations. Liver samples were obtained and processed for proteomic analysis, histopathological analysis and for antioxidant enzymatic analysis (MDA, CAT, SOD, GPX and pNPB).

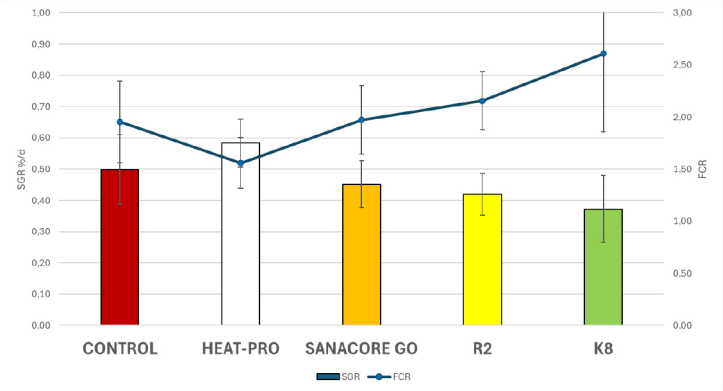


**Figure 1.** SGR and FCR values of seabass during the recuperation period (10 days) after the mortality caused by a heat stress period (one week at 32ºC).

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**Results & conclusions**

No difference among diets were found in SGR nor FCR during the 9 weeks that the seabass was cultured at 28ºC. However, after one week at 32ºC, mortality appeared in all the fish fed the experimental diets. Seabass fed diet HeatPro diet presented the highest survival, followed by the fish fed diet Sanacore and control diet (95,24%, 92,86% and 88.10 % respectively). After the mortality episode, fish was kept in the system for 10 more days at 28ºC. Although no significant difference was found for SGR nor FCR, seabass fed diet Sanacore showed a 17% better SGR and 20% lower FCR than the fish fed the control diet (Figure 1).

Plasma metabolites showed significant differences for cortisol, glucose and lactate (lowest values for fish fed the HeatPro diet). Histopathology analysis showed that all the fish were highly affected by the temperature increase during the trial; all groups presented a marked level of intracytoplasmic lipid-type vacuolation in the liver and the high temperature may have promoted direct or indirect liver damage at subclinical level. Enzymatic antioxidant analysis pointed out that fish fed Sanacore diet had significantly lower MDA values. No difference was found for CAT, SOD, GPX or pNPB, although fish fed Sanacore showed the lowest values, except for pNPB. Molecular (stress markers) analysis of the fish fed with HeatPro diet showed reduced HSP70 and PRDX3 level, indicating lower cellular stress, and increased IGF-1 expression, reflecting improved recovery and metabolic balance.

As conclusion of the study, it can be pointed out that seabass subjected to heat stress (temperature above 30ºC) and fed HeatPro diet showed better SGR, FCR, cortisol, lactate and glucose plasma levels than the fish fed control diet. On the other hand, fish fed Sanacore diet showed significantly lower MDA values than the fish fed control diet. Molecular findings suggest that the supplementation with HeatPro enhances stress tolerance and physiological resilience in seabass under high-temperature conditions.

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# BIOCHAR AS A PROMISING FEED ADDITIVE IN DIETS FOR ATLANTIC SALMON PRE-SMOLTS

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## Introduction

The use of biochar as a feed additive has gained increasing attention in animal nutrition due to its potential benefits on digestion, nutrient absorption, and overall health. Biochar, a carbon-rich product derived from pyrolysis of organic materials, has been traditionally used in animal husbandry for toxin adsorption and digestive health enhancement, but recent studies have highlighted its broader applications, including improvements in feed efficiency, reduction of greenhouse gas emissions, and enhancement of animal welfare (Schmidt et al., 2019). However, the systematic evaluation of biochar's effects on aquaculture species, particularly Atlantic salmon, remains limited.

## Experimental set-up

A basal commercial-like diet (D1) was formulated to meet or exceed the nutritional requirements of Atlantic salmon according to NRC (2011), without any additional growth-promoting ingredients (Table 1). Biochar, either 0.25% (D2) or 0.50% (D3), was added to the basal diet. The biochar was provided by OBIO (Norway) and was ground to 1mm.

The diets were produced by extrusion at Fôrtek (Center for Feed Technology, NMBU, Aas, Norway), with a 2mm die and with a final diameter of ca. 2.4mm. After drying until 8-10% moisture, pellets were vacuum coated with fish oil and rapeseed oil.

A four-week experiment was conducted at the Center for Fish Trials (NMBU, Aas, Norway). A total of 315 pre-smolts Atlantic salmon with an initial average body weight of 31.7 g were randomly distributed into nine 300L fiberglass tanks (35 fish per tank). Fish received one of the three diets, *ad libitum*, with three replicate tanks per diet. Each tank had a capacity of 300 L. Uneaten feed was collected daily to calculate feed intake according to Helland et al. (1996).

## Results and Discussion

Physical pellet quality: The inclusion of biochar in diets for Atlantic salmon significantly affected the physical pellet quality. Pellets from diets D2 and D3 were harder ($p = 0.001$) and had slower sinking velocity ($p = 0.012$) compared to D1 pellets, which allowed the pellets to stay in the water column longer, providing more time for the fish to feed and thereby reducing feed waste. In addition, diet D3 had a significantly higher water stability index ($p = 0.039$), which is a desired characteristic for fish production in RAS systems.

Lastly, in accordance with the previous results, there was a lower waste output in fish fed diets containing biochar (Figure 1). There was a strong tendency for reduced total solid waste ($p = 0.065$) and a significant reduction in solid nitrogen waste ($p = 0.015$), suggesting that biochar improves nutrient utilization, potentially reducing nitrogen excretion. Overall, results suggest that the use of biochar in RAS systems can reduce solid wastes, reduce maintenance costs, and minimize environmental impact.

Growth performance: No significant differences were observed in the growth performance parameters such as specific growth rate (SGR), feed conversion ratio (FCR) and final body weight. However, there was a numerical increase in weight gain and specific growth rate (SGR) and a numerical decrease in FCR for fish fed diets D2 and D3 compared to D1.

Digestibility: The apparent digestibility coefficients of dry matter, crude protein, ash, and carbon increased significantly in fish fed diets D2 and D3 (Table 2). This improved digestibility suggests a better nutrient utilization when Atlantic salmon diets are supplemented with biochar.

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Table 1. Diet composition of D1

| Ingredients | (g/kg) |
|---|---|
| Fish meal | 244.4 |
| Soybean meal | 290.0 |
| Wheat gluten meal | 80.0 |
| Pea protein concentrate | 45.0 |
| Wheat flour | 120.0 |
| Rapeseed oil | 60.0 |
| Fish oil | 120.0 |
| Monocalcium phosphate | 20.0 |
| Vitamin and mineral premix | 5.0 |
| L-lysine | 9.0 |
| DL-methionine | 3.0 |
| L-threonine | 1.0 |
| Choline chloride | 1.5 |
| Yttrium oxide | 0.1 |
| Iron | 1.0 |

Table 2. Apparent digestibility coefficient (%) in Atlantic salmon (n=3 tanks per diet).

| ADC % | D1 | D2 | D3 | SEM | $P_{ANOVA}$ |
|---|---|---|---|---|---|
| Dry matter | $72.70^{A}$ | $74.55^{B}$ | $75.33^{B}$ | 0.43 | 0.0045 ** |
| Ash | $13.47^{A}$ | $19.83^{B}$ | $21.25^{B}$ | 1.26 | 0.0011 ** |
| Crude protein | $91.48^{A}$ | $92.76^{B}$ | $92.89^{B}$ | 0.26 | 0.0201* |
| Carbon (C) | $79.31^{A}$ | $80.22^{AB}$ | $80.98^{B}$ | 0.27 | 0.0094** |

Intestinal health and metabolism: Histological analysis of 12 fish/diet revealed that the inclusion of biochar did not have any effect on mucosal structure. No significant differences in enteritis or mucosal atrophy were observed. Analysis of plasma revealed no differences between dietary groups regarding their enzyme activity (e.g., ALP, AST, DGGR), metabolites (e.g., albumin, bile acid, glucose, cholesterol), vitamins (A, D and E) or minerals (K, Ca, Na, P). Biochar is commonly associated with a high absorption capacity and there was the possibility of some nutrients being retained by the biochar and not absorbed by the fish. The current results suggest that biochar did not impact intestinal structure nor plasmatic levels of metabolism-related compounds.

Microbiota: 16S rRNA sequencing in distal intestine digesta is currently being carried out to determine the effect of biochar in intestinal microbiota, as has been reported in other studies.

**Conclusion**

The current results suggest that biochar has potential as a sustainable feed additive in RAS aquaculture. Inclusion of biochar in diets not only reduced solid waste production, potentially improving system efficiency, but also resulted in improved nutrient utilization and had no adverse effects on fish health.

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# IS *Phaeobacter inhibens* ABLE TO CONTROL *Vibrio* IN *Artemia salina* LIVE FEED CULTURE*?*

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## Introduction

Live-feed cultures are a key vector of *Vibrio* contamination in larviculture. *Phaeobacter sp.* has been suggested as good biocontrol candidates by biosynthesis of the antibacterial secondary metabolite tropodithietic acid (TDA).

## Material and Methods

An *Artemia salina* trial was performed in a commercial facility within 5L static tanks. The trial tested three incremental doses of *P. inhibens* (live freeze-dried powder form; Lallemand, France) in triplicate against a Control. The test product was applied in the rearing media at the start of both the hatching- and the enrichment phases using LARVIVA Multigain (BioMar). Artemia rearing remained otherwise standard and followed commercial practices. At the end of both the hatching- and rearing-phase; artemia and water samples were collected to quantify *Vibrio* load in TCBS agar media and to perform 16S rRNA amplicon sequencing by Miseq, then analysed with DADA2 in QIIME2.

## Results

The addition of *P. inhibens* significantly reduced the artemia *Vibrio* load at the end of enrichment. The higher doses tested clearly modulated the composition of the artemia microbial community which was concomitant with a significantly higher Shannon-index. The relative abundance of *Phaeobacter* increased (and other interesting genera as *Exiguobacterium*) and of *Vibrio* decreased, with increasing inputs of *P. inhibens*. The Rhodobacteraceae/Vibrionales ratio was confirmed as a relevant index also in *Artemia salina* culture. This first *in vivo* trial using a live, freeze-dried form of *P. inhibens* confirms this candidate agent as a potential and practical *Vibrio* biocontrol strategy in marine fish hatchery.

# EFFECTS OF DIFFERENT PLANT-BASED DIETS ON THE FATTY ACID COMPOSITION OF THE MARINE AMPHIPOD *Gammarus locusta*

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## Introduction

The use of sustainable aquafeed ingredients in aquaculture primarily aims to reduce pressures on marine ecosystems by minimizing the reliance on fish meal and fish oil production. Most sources of omega-3 fatty acids (FA) come from highly exploited marine resources; however, omega-3 FA display a pivotal role in marine aquaculture. In the search for environmentally friendly and economically viable feed ingredients, terrestrial plants sources have emerged as promising alternatives to traditional marine-based inputs, but feature several limitations (e.g., limits of incorporation, anti-nutritional effects…). The present study evaluated the effects of different plant-based diets on the fatty acid profile of the marine amphipod *Gammarus locusta*, a key species in marine trophic chains and a potential candidate for inclusion in marine aquafeeds due to its content in long-chain polyunsaturated fatty acids (PUFA). This approach aims to further merge green and blue food ecosystems.

## Material and methods

Offspring of *G. locusta* were fed with four different diets: *Ulva* sp. (Ul) (used as control), peas (Pe), green beans (GB), and carrots (Ca), until reaching sexual maturity. Subsequently, individuals were collected, and their fatty acid composition determined. Fatty acid methyl esters were analysed via gas chromatography- mass spectrometry (GC-MS). The absolute abundance of fatty acids was calculated in $\mu$g/mg dry weight (DW), and data were compared across different diets.

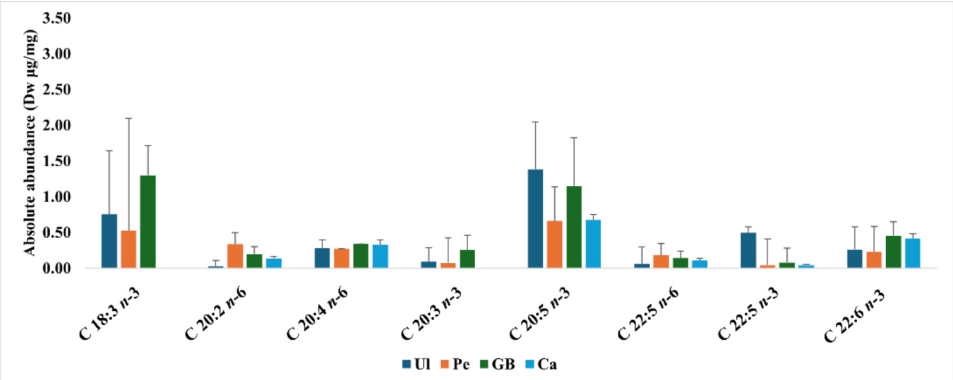


**Figure 1** – Mean abundance of polyunsaturated fatty acid composition of *Gammarus locusta* supplied with *Ulva* sp. (Ul), pea (Pe), green bean (GB) and carrot (Ca). Data expressed as µg.mg$^{-1}$ DW and representing the mean ± SE of 5 replicates ($n$ = 5), with exception for carrot (n=3).

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**Results**

The fatty acid composition of *G. locusta* clearly reflected their diets. Amphipods fed *Ulva* sp. showed higher levels of eicosapentaenoic acid (EPA) (C20:5*n*-3) (Ul: 1.39 ± 0.15, Pe: 0.70 ± 0.18, GB: 1.15 ± 0.32 and Ca: 0.68 ± 0.09, $\mu$g/mg DW) (Figure 1). However, in the case of docosahexaenoic acid (DHA) (C22:6*n*-3), amphipods fed *Ulva* displayed lower values when compared to conspecifics fed plant-based diets (Ul: 0.26 ±0.07, Pe: 0.28 ± 0.09, GB: 0.46 ± 0.08 and Ca: 0.41 ± 0.08 $\mu$g/mg DW) (Figure 1). It is worth highlighting that amphipods fed on terrestrial plant diets (Pe, GB, Ca) exhibited both EPA and DHA, despite the absence of these fatty acids in their diets

**Discussion**

These findings highlight the metabolic plasticity of *G. locusta* and its potential ability of synthesising long-chain omega-3 PUFAs, such as EPA and DHA from diets without these fatty acids. Although marine ingredients like *Ulva* sp. can directly supply EPA (in residual amounts), terrestrial diets enable biosynthesis of EPA and DHA, offering a sustainable strategy to farm *G. locusta*, whose biomass can eventually be used as an ingredient/supplement for aquafeeds. This approach is a promising pathway for converting low-cost, terrestrial inputs into high-value marine ingredients through bioconversion.

**Conclusion**

*G. locusta* can biosynthesise essential *n*-3 PUFAs (namely EPA and DHA) from terrestrial plant-based diets, making them viable and sustainable inputs for producing enriched live feeds. The use of terrestrial diets represents a novel and ecologically sound approach for generating DHA and EPA through trophic upgrading.

**Acknowledgements**

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# METHODOLOGICAL FRAMEWORK FOR ENVIRONMENTAL SUSTAINABILITY ASSESSMENT OF FRESHWATER AQUACULTURE SYSTEMS: THE CASE OF THE SAFE-SMARTAQUA4FUTURE PROJECT

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## Introduction

Aquaculture is the fastest growing food production system worldwide, having increased from 10 million tonnes in 1990 to 94.4 million tonnes in 2022 (FAO, 2024). While global aquaculture production continues to grow rapidly, the EU freshwater aquaculture sector has largely stagnated over the past two decades (Guillen *et al.*, 2025). This sector faces significant challenges including feed costs, environmental concerns such as nutrient loading and resource use in addition to inefficient waste management practices that fail to capitalize on circular economy opportunities. The SmartAqua4FuturE (SAFE) project aims to reverse the declining trend in European freshwater aquaculture through novel circular economy approaches to waste stream valorisation. Central to this initiative is the development of a comprehensive environmental sustainability assessment framework that can evaluate the environmental performance of different freshwater aquaculture systems (FWAS) and SAFE innovations. The project will evaluate the environmental performance of a number of approaches that can reduce the impacts of aquaculture – these include novel ingredients for feed production, approaches to aquaculture operation and methods for enabling valorisation of aquaculture waste. This abstract presents the work developed towards the advancement of a standardised methodological framework for conducting Life Cycle Assessment (LCA) of FWAS, with particular emphasis on integrating biodiversity elements. By developing these novel approaches, this work will contribute to the development of an LCI framework that can be extensively adopted by scientific organisations, industry and aquaculture operators. The boundaries of the analysis for these approaches are also presented.

## Methods

System boundaries were established for eight systems: carp polyculture in China, carp monoculture in Poland, flowthrough trout farming in Portugal, RAS/pond perch farming in Ireland, RAS salmonid farming in Denmark, and three SAFE innovations (mushroom cultivation, redworm meal production, and mealworm production). For each system, appropriate functional units were considered, such as kg of fish at the farm gate. The environmental sustainability assessment methodology follows ISO 14040/14044 standards for LCA and is aligned with EU Product Environmental Footprint Category Rules (PEFCR) and Environmental Product Declaration (EPD) guidelines.

The assessment covers three main systems: 1) EU FWAS including extensive pond-based operations and intensive recirculating aquaculture systems (RAS), 2) feed trials examining both conventional and novel feeds incorporating circular alternative ingredients and 3) SAFE innovations for waste stream valorisation. A structured data collection framework was developed to ensure methodological consistency and data quality. Primary data will be collected through tailored surveys targeting farm-level inputs (feed, energy, water, chemicals) and outputs (fish production, waste streams), while secondary data from scientific literature and databases will complement background processes. Several The pedigree matrix approach was incorporated to facilitate transparent assessment of data quality. Environmental impact categories selected for assessment include carbon footprint, acidification potential, eutrophication potential, freshwater consumption, land use requirements, and biotic resource use. Additionally, FWAS-specific indicators such as Fish In Fish Out (FIFO) ratio will be included to provide sector-relevant performance metrics. A novel aspect of the methodology is the integration of biodiversity indicators into the LCA framework through three complementary strategies: the Water Framework Directive (WFD) methodology, to assess impacts on water body ecological status; the integration of locally relevant species that could serve as context-specific bioindicators such as such as benthic macroinvertebrates and aquatic macrophytes, that reflect the unique ecological conditions of different aquatic ecosystems, through a structured self-reporting system for farmers to document species observed at their facilities; and lastly, the incorporation of land use metrics to assess effects of land use on water bodies health and aquatic biota.

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## Results and Discussion

The methodological framework presented establishes a foundation for comprehensive environmental sustainability assessment of freshwater aquaculture systems and innovations developed within the SAFE project. By adhering to standardized LCA protocols while adapting them to the specific challenges of aquaculture production, this framework enables meaningful comparison across different production systems and technologies. Preliminary work on integrating biodiversity indicators represents a significant advancement in addressing a fundamental gap in the environmental sustainability assessment of the aquaculture sector. The proposed approach combining WFD methodologies, bioindicator species monitoring, and land use metrics offers a more holistic evaluation of biodiversity impacts than the conventional LCA approaches, which typically focus solely on land use change.

The methodology developed will enable the evaluation of environmental performance and potential trade-offs associated with circular economy innovations in freshwater aquaculture. For instance, valorising aquaculture waste streams for novel feed ingredients might reduce dependence on marine-derived ingredients and lower the overall environmental footprint but could potentially introduce new challenges related to processing energy requirements or nutrient profiles.

## Conclusion

The standardized methodology developed within the SAFE project provides a robust framework for assessing the sustainability of freshwater aquaculture systems and circular economy innovations. By integrating conventional LCA approaches with aquaculture-specific indicators and enhanced biodiversity assessment, this methodology addresses key gaps in sustainability evaluation for the sector. When fully implemented, this framework will inform evidence-based decisions regarding the environmental performance of different production systems and technologies, ultimately supporting the European freshwater aquaculture sector's transition toward more sustainable and circular production methods. The approach developed has broader applications beyond the SAFE project and could contribute to standardizing environmental sustainability assessment methodologies for the global aquaculture sector.

## Acknowledgments

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# APPLICATION OF HYPERSPECTRAL IMAGING FOR THE CLASSIFICATION AND CHARACTERIZATION OF STURGEON CAVIAR AND TROUT ROE

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## Introduction

The classification of a product such as caviar is crucial for ensuring its quality, authenticity, and market value. For Caviar Pirinea, this categorization is especially significant due to its unique characteristics and distinctive origin. Selecting the appropriate category helps differentiate between various qualities and types, protects consumers, and enhances the product's value both locally and internationally [1, 2] Additionally, proper classification helps preserve the tradition and sustainability of caviar production, both of which are essential for maintaining its reputation and competitiveness. [3]. The same applies to trout roe, which also needs proper classification and description to guarantee its quality and freshness and to increase its value in the gourmet market. Although trout roe is less expensive than sturgeon caviar, it is appreciated for its taste and uses in cooking. Clear categories help improve how it is sold and protect the consumer [4, 5].

Hyperspectral imaging is an advanced technology that allows rapid and non-destructive analysis of food, capturing detailed information about its chemical composition and physical characteristics. This technique uses multiple wavelengths to obtain images that reveal properties invisible to the human eye, facilitating the detection of defects, contaminants, or variations in product quality [6, 7]. Its application in food quality control is growing rapidly, as it provides accurate results and helps ensure safety and freshness, thereby increasing consumer confidence and optimizing production processes [8].

## Materials and methods

The hyperspectral imaging cameras used for this experiment were the FX10 (Specim, 400–1000 nm) and the FX17 (Specim, 900–1700 nm). Due to the glare and reflections caused by the product matrix itself, a diffuse lighting system was required to capture high-quality images.

For this study, samples from three sturgeon caviar varieties were selected: Baerii, Naccarii, and Osetra. As for the trout roe, all samples originated from rainbow trout. A total of 80 sturgeon caviar samples and 50 trout roe samples were analyzed.

The primary objectives of product classification were to assess the color, size, moisture, and firmness of sturgeon caviar and to separate trout roe into high and lower quality categories, and to measure salt content.

The methodology followed was based on the following work-flow: hyperspectral image acquisition, data processing to differentiate and ROI selection, extraction of the average spectrum per ROI or sample, and development of regression or classification models depending on the parameter of interest.

## Results and discussion

After applying various approaches and calibrating models according to the parameters of interest for each product, it was observed that the FX17 hyperspectral camera delivered better results than the FX10 for most of the evaluated parameters.

For sturgeon caviar, the models developed with the FX17 to estimate diameter and moisture content showed the best performance, outperforming the models related to firmness and color. The limited availability of samples in one of the firmness classes reduced the robustness of the results, making them inconclusive. Regarding color, the models were able to distinguish between light, dark, and bi-colored roe; however, the classification of intermediate shades could not be adequately trained with the available sample set.

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For trout roe, both quality classification (high or low) and salt content prediction yielded good results with both cameras. Nevertheless, it is important to note that the image quality of the FX10 is lower than that of the FX17, which significantly reduces the number of roe identified per sample. Therefore, the results obtained with FX17 are more representative.
Overall, the results indicate that the FX17 hyperspectral imaging camera is a highly promising technology for the classification and categorization of both sturgeon caviar and trout roe.

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# EFFECTS OF OCEAN ACIDIFICATION ON GROWTH AND MINERALIZATION OF SKELETAL STRUCTURES IN GILTHEAD SEA BREAM (*Sparus aurata*)

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## Introduction

The increase in anthropogenic carbon dioxide ($CO_2$) emissions results in a decrease in oceanic pH, a phenomenon known as ocean acidification (OA), which represents a considerable risk to the future of aquaculture. In marine invertebrates, OA reduces survival, growth, and calcification. In fish, elevated $CO_2$ levels in water have also been associated with reduced survival, specific growth rate (SGR) and protein biosynthesis, as well as alterations in behavior and reproduction (Heuer and Grosell, 2014). Contrarily, OA has been reported to enhance otoliths growth and calcification, as well as to modify their shape and composition, possibly due to efficient plasma pH compensation mechanisms. In fish with cellular bone, changes in bone mineralization under hypercapnic conditions have also been reported (Drábiková et al., 2023; Gil Martens et al., 2006). Furthermore, *in vitro* studies in mammals have demonstrated that osteoblasts activity is significantly affected by low pH conditions. However, the impact of $CO_2$ on fish with acellular bone mineralization remains less understood. The aim of this study is to assess the potential effects of OA on the skeleton in gilthead sea bream juveniles, using both *in vivo/in vitro* approaches.

## Material and methods

Gilthead sea bream juveniles provided by the commercial hatchery Piscimar (Burriana, Spain) were maintained in the animal facilities at the Faculty of Biology at the University of Barcelona (Spain). After an acclimation period, animals were weighed and randomly distributed in two experimental groups exposed to different pH conditions: a control group maintained at pH 7.9, and a low pH group maintained at pH 7.3 by $CO_2$ injection through a glass diffusor connected to a $CO_2$ bottle. After 68 days of exposure, fish were measured and weighed, and 15 fish per condition were sampled. Blood was collected to determine hematocrit levels and plasma composition. Otoliths, gills and vertebral bone were carefully extracted and immediately frozen in liquid nitrogen. Bone mineral composition and mineralization by X-ray, remodeling and mineralization-related genes expression, as well as ion transport gene transcription in gills, were analyzed. Furthermore, primary cultures of gilthead sea bream bone-derived cells were performed. Then, cell viability and mineralization were assessed on cells cultivated under three different conditions, 2.5%, 1.5%, or 0% $CO_2$, corresponding to a pH value of 7.79 ± 0.04, 7.49 ± 0.01, and 7.38 ± 0.02, respectively.

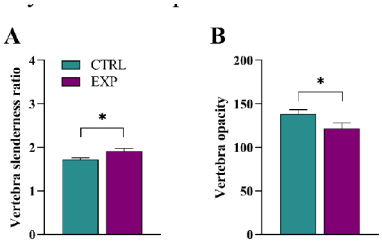


**Figure 1. (A)** Slenderness ratio and **(B)** opacity of the vertebrae of gilthead sea bream juveniles exposed to control (CTRL) pH (7.9) and experimental (EXP) pH (7.3) for 68 days. Data are shown as Mean + SEM ($n$ = 15). Statistical differences were determined by student-unpaired t-test ($p$<0.05).

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**Results and discussion**

No significant differences were observed in body weight or body length at the end of the experimental period. However, the SGR values were significantly lower in the experimental group compared to the control group, consistent with findings in European sea bass (*Dicentrarchus labrax*) (Alves et al., 2020). Plasma analysis revealed significantly higher pH and potassium levels in the experimental group, while ionic calcium, magnesium, and phosphorous levels remained unaltered, in agreement with previous studies (Alves et al., 2020; Costa et al., 2022). Bone mineral composition did not show significant differences between groups for any of the ions evaluated. However, X-ray analysis indicated an increase in vertebra slenderness ratio and a reduction in opacity in the experimental group (Figure 1). This suggests that, despite being less mineralized than in the control group at this time, the vertebrae of animals exposed to low pH appear to have undergone more longitudinal growth and could potentially reach higher mineralization levels if given a longer growth period. Otoliths from fish exposed to low pH exhibited a generalized increase in size, with a significant increase in most of the parameters determined compared to the control pH group. No significant differences were identified in the transcription levels of gills ion transporters between groups. Nevertheless, the low pH condition significantly upregulated the expression of the osteoblast marker, *alkaline phosphatase* (*alp*), and the osteoclast-related gene, *matrix metalloproteinase-9* (*mmp9*), suggesting increased bone turnover in the experimental fish. In the *in vitro* experiments, the percentage of $CO_2$ had a significant effect on both cell viability and mineralization, with the highest values for those parameters observed under the 0% $CO_2$ condition after 30 days. Overall, the results of the present study provide new evidence of how climate change, particularly OA, can impact the skeletal structures of gilthead sea bream, an important species for both Mediterranean ecosystems and aquaculture industries.

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# OAK BIOCHAR AS A SUSTAINABLE ADDITIVE IN AQUAFEEDS FOR SENEGALESE SOLE (*Solea senegalensis*): EFFECTS ON GUT MICROBIOME

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## Introduction

Biochar, a carbonaceous material derived from the thermochemical conversion of biomass under oxygen-limited conditions, has emerged as a versatile material with applications in environmental remediation, including the sequestration of heavy metals, organic pollutants and other hazardous substances. In recent years, the supplementation of aquatic feeds with biochar has shown potential to stimulate growth and development in various aquatic organisms (Rai et al., 2025). In this study, two experimental diets supplemented with biochar GM-530-P, produced industrially from cork oak and holm oak stumps at 530 ºC, were evaluated after 5 months of administration in *Solea senegalensis* juveniles.

## Material and methods

Experimental diets were formulated according to the nutritional requirements of the species *Solea senegalensis*. A control diet (CT) and two experimental diets supplemented with 0.5% (B0.5) and 2% (B2) of biochar GM-530-P were assayed. A total of 135 fish (average length of 14.46±0.5 cm and weight of 40.93±4.01 g) were randomly distributed across 9 tanks. Fish were reared for 5 months at the Marine Aquaculture Facility "El Bocal" of the Spanish Institute of Oceanography (Santander), and sampled monthly to monitor growth. At the end of the trial, intestines were aseptically dissected. Mucosal samples from the anterior and posterior intestinal sections were collected in sterile microtube, and stored at –80 °C until further analysis. DNA metabarcoding library preparation and sequencing were carried out by AllGenetics & Biology SL (www.allgenetics.eu).

## Results and discussion

The microbial community structures were successfully described at various taxonomic levels, including phylum, class, order, and family. Analysis of the alpha diversity in the anterior intestine revealed that B05 diet generally led to significantly greater microbial richness compared to both the B2 and CT diets. Also, the fish fed B05 diet demonstrated significantly higher overall diversity, as indicate by a higher Shannon index and a lower Simpson index. In contrast, the posterior intestine samples exhibited less pronounced differences, with no statistically significant variations in alpha diversity indices among the B0.5, B2, and CT dietary groups.

Graphical representations of microbial relative abundance models, highlighting differentially abundant taxa at the family level, are shown in Figure 1.In the anterior gut (Figure 1A), both biochar-supplemented diets (B05 and B2) consistently induced significant shifts in the microbial landscape relative to the control (CT). A notably increased relative abundance was observed for families such as *Alteromonadaceae, Vibrionaceae, Thiohalorhabdaceae, Halieaceae, Nitrosomonadaceae, Rhodobacteraceae*, and members of the *SAR116 clade,* all showing positive associations with biochar inclusion ($p < 0.05$). These groups often include opportunistic colonizers or taxa involved in various metabolic processes, including sulfur and carbon cycling. In contrast, the posterior gut (Figure 1B) displayed a more nuanced and compartment-specific response to biochar supplementation. While some families, like *Vibrionaceae, Thiohalorhabdaceae,* and *Nitrosomonadaceae*, showed consistent increases in abundance across both gut sections, other prominent changes were unique to the posterior segment. Notably, significant reductions in relative abundance were observed for *Desulfocapsaceae,* certain *Actinobacteriota*, and *Flavobacteriaceae*.

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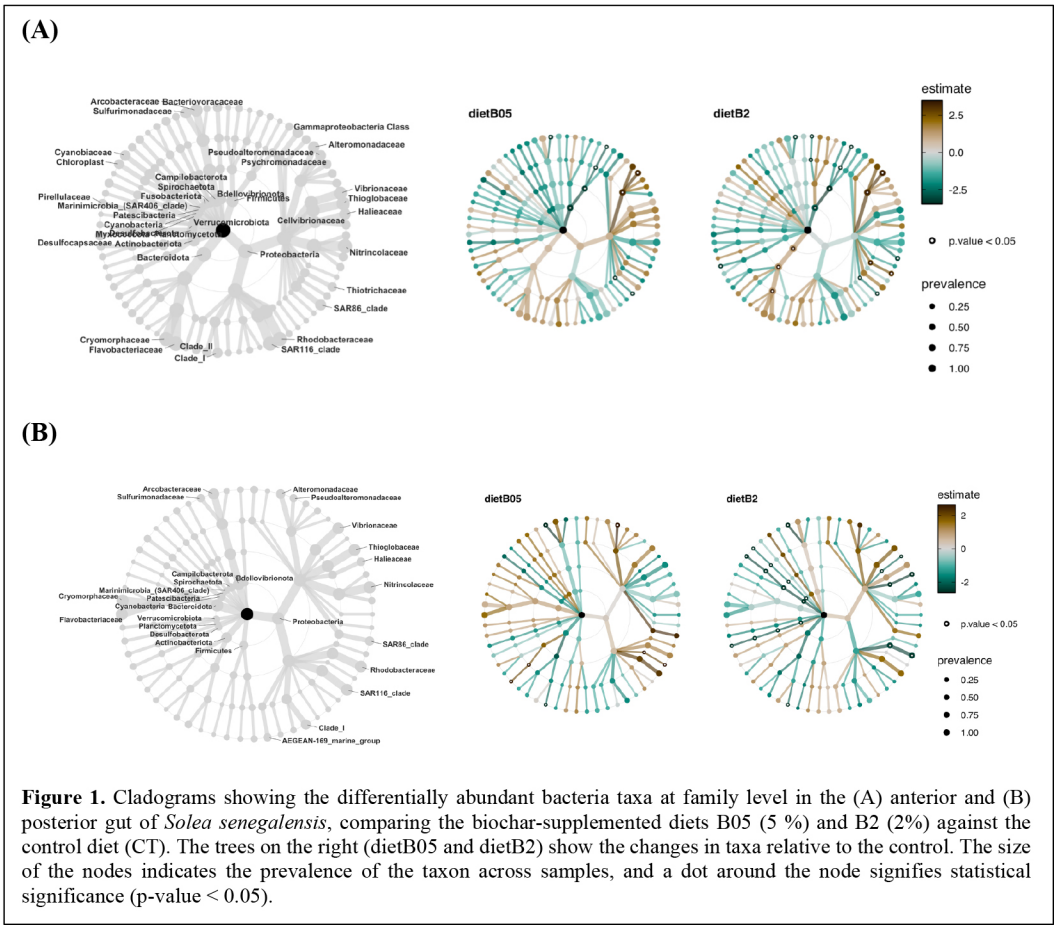


**Figure 1.** Cladograms showing the differentially abundant bacteria taxa at family level in the (A) anterior and (B) posterior gut of *Solea senegalensis*, comparing the biochar-supplemented diets B05 (5 %) and B2 (2%) against the control diet (CT). The trees on the right (dietB05 and dietB2) show the changes in taxa relative to the control. The size of the nodes indicates the prevalence of the taxon across samples, and a dot around the node signifies statistical significance (p-value < 0.05).

The observed microbial shifts have potential implications for Senegalese sole aquaculture. An increased diversity in the anterior gut, particularly in fish fed B0.5 group, could enhance digestive efficiency and nutrient absorption. Alterations in specific metabolic groups (e.g., sulfur and nitrogen cycling bacteria) may influence gut functionality and the production of beneficial or detrimental metabolites. Further research is warranted to elucidate the functional consequences of these taxonomic shifts on host physiology, nutrient utilization efficiency, disease resistance, and growth performance.

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# POSITIVE BEHAVIORAL AND WELFARE EFFECTS OF STRUCTURAL ENRICHMENT ON CAPTIVE EUROPEAN SEABASS

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## Introduction

The increasing demand for protein by the human population has driven the growth of the aquaculture industry, with fish being the most widely farmed organisms. Nevertheless, captive conditions are often inadequate due to spatial uniformity, homogeneity, and the immutability of the environment. These conditions can impair the welfare and cognitive abilities of the fish 1,2. In this context, providing environmental enrichment (EE) is a well-established strategy to promote animal welfare by offering novel stimuli that support behavioral, physiological, and psychological needs 1,3. In aquatic species, structural EE, understood as the addition of physical complexity through objects or structural modifications 1, has been shown to reduce stress, enhance cognitive abilities, and mitigate abnormal behaviors 4–8. The effects of EE vary depending on the species and its life stage1,8. Despite the growing interest in the effects of this strategy in captive fish, there remains a significant lack of knowledge regarding some of the most relevant species for the industry, such as the European seabass (Dicentrarchus labrax, L.)3. Consequently, our aim was to analyze the effects of environmental stimulation through physical structures, considering behavioral, physiological, and cognitive aspects in European seabass, to assess their potential applications for improving welfare.

## Material and Methods

The study was carried out in experimental facilities located in the coastal waters of Port of Andratx (Balearic Islands, Spain), belonging to the Laboratory of Marine Research and Aquaculture (LIMIA-IRFAP). A total of 4000 juvenile seabass of about 30 g in weight, were randomly distributed into 8 experimental marine sea-cages. Four of these sea-cages were structurally enriched, while the remaining four were kept bare, serving as the control (EE and NE cages, respectively). The applied structural EE consisted of a floating rectangular cork from which six knotted ropes with a weight at the end were suspended, occupying more than half of the vertical space within the sea-cage (1.5 m). The juvenile individuals were maintained under the influence of the treatments for approximately 3.5 months (03/11/2024 - 07/05/2024; 116 days).

After 2.5 months from the beginning of the enrichment period, the emotional reactivity test (ERT) was conducted over four consecutive days to evaluate the effects of structural EE on the behavioral response of seabass, testing ten individuals per marine sea-cage. For this behavioral test the fish was placed into a circular experimental tank (Ø 55 cm, 38 cm high) that it had never explored before (Open-field test). The researcher then leaved the vicinity, marking the start of the ERT, during which behavioral responses of each fish were recorded individually for 10 minutes with a top-view camera (GoProHero10®). At the end of the testing period all individuals were euthanized with an overdose of anesthetic and subsequently sampled. Muscle and telencephalon tissues were dissected and immediately frozen at –80 °C for subsequent analysis of oxidative status in the laboratory. The activity of three antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GRd)] was quantified, along with the concentration of malondialdehyde (MDA), a compound commonly used as a marker of membrane phospholipid oxidation.

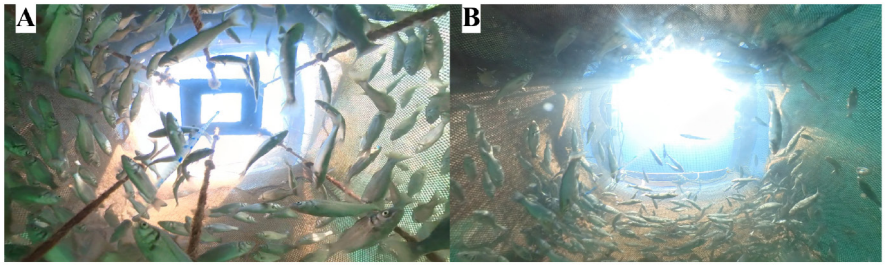


**Figure 1.** Underwater view with 360º cameras of the EE cages (A) and the NE cages (B)

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**Results and Discussion**

Results showed that fish reared under environmental enrichment displayed enhanced exploratory behavior, covering greater distances, and utilizing a larger area more uniformly across the available space. Furthermore, the day-to-day variations observed in the different parameters of the ERT in enriched fish were consistent with a normal habituation process to a novel environment, indicative of positive welfare. In contrast, control fish exhibited higher anxiety levels and lacked such habituation patterns. Overall, enriched fish demonstrated more active behavior and greater adaptability to unfamiliar situations, whereas non-enriched fish showed limited exploration and a more constrained response. Additionally, this behavioral pattern was associated with the activation of muscle enzymatic mechanisms that prevented oxidative damage, reflecting greater physiological resilience under challenging conditions. These findings highlight the role of EE in fostering positive welfare states in fish by promoting natural behaviors and reducing stress responses. The implementation of structural EE represents a promising, non-invasive approach to improving fish welfare in captivity or controlled environments, warranting further investigation and potential applications.

**Acknowledgements**

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# META-QTL ANALYSIS FOR PATHOGEN RESISTANCE IN RAINBOW TROUT

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## Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most extensively cultivated freshwater fish species, with a global production reaching 900 thousand tonnes per year (FAO, 2024). However, this species is vulnerable to a range of diseases that present major challenges to animal health and welfare, which negatively impacts on aquaculture production and results in significant economic losses for aquaculture farms (Kania & Buchmann, 2025). To mitigate these adverse effects, genetic improvement can be accelerated through quantitative trait loci (QTL) mapping. This approach has enabled the identification of genomic regions associated with disease resistance in rainbow trout (Buchmann, 2022). Although numerous QTLs associated with pathogen resistance have been reported in rainbow trout, differences among studies, such as experimental design, fish populations, trait selection, statistical methods and the quality and density of the genetic map, make it challenging to identify QTLs for all relevant traits within a single analysis. To address these challenges, meta-analysis supported by mathematical models has emerged as an effective strategy to improve the accuracy and reliability of QTL localization, as it allows for the integration of data from multiple studies (Kaur et al., 2023). To the best of our knowledge, this study represents the first meta-analysis of pathogen resistance QTLs in rainbow trout, highlighting a major research gap that could improve disease-resistant breeding programmes in aquaculture.

## Material and Methods

A comprehensive literature review was conducted to collect QTLs associated with pathogen resistance in rainbow trout, covering studies from the last two decades. Key data including population mapping type, population size, chromosome number, QTL and flanking markers positions, LOD score, and explained variance were extracted. The consensus map for meta-QTL analysis was constructed using the R package "LPmerge" and six genetic maps. QTL positions were projected onto this consensus map, and 95% confidence intervals were calculated with population-specific formulas: For $F_2$ generation and Backcross populations: CI (95%) = $530/(R^2 \times N)$; for doubled haploid populations: CI (95%) = $287/(R^2 \times N)$, where $R^2$ is the phenotypic variance explained by the individual QTL and $N$ is the population size. Meta-analysis of QTLs was performed for each chromosome using Biomercator v4.2.3, applying the Goffinet & Gerber's method for chromosomes with ≤10 QTLs and the Veyrieras et al.'s method for those with >10 QTLs.

## Results

A total of 138 QTLs associated with pathogen resistance traits were available for meta-QTL analysis. The number of initial QTLs per chromosomes ranged from a minimum of 1 QTLs on chromosome 9, 14,15,16 and 17 to a maximum of 21 QTLs on 8 (Fig 1). Most QTLs (88.40% of total) had LOD score between 2 and 3. The phenotypic variation explained by individual QTL varied from <5 to 30> with an average of 15.16%, suggesting the involvement of both major and minor QTLs in governing pathogen resistance.

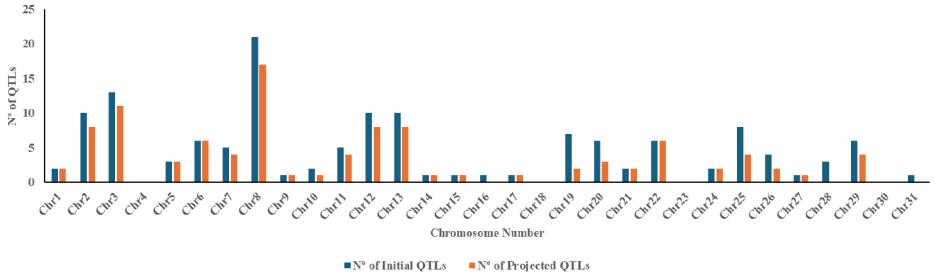


**Fig 1.** Distribution of initial and projected QTLs for Meta-QTL analysis.

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The high-density rainbow trout consensus map generated in present study, using six different genetic maps, exhibited an average marker density of 2.84 markers/cM across the genome. Moreover, there were approximately 513 markers mapped on average per chromosome. Out of 138 QTLs, a total of 101(73.19%) could be projected of the total onto the newly constructed consensus map (Fig. 1). The method proposed by Gerber and Goffinet was employed for meta-analysis of QTLs on all chromosomes except for chromosome 3 and 8, for which the Veyrieras approach was used. A total of 26 meta-QTLs (MQTLs) were predicted for resistance to pathogens. Across the whole genome, the maximum number of MQTLs was predicted on chromosome 8.

**Discussion**

The quantitative genetics of pathogen resistance has been explored in numerous studies, mainly because of the molecular markers availability, lower genotyping expenses, and advances in QTL mapping techniques (Huang et al., 2025). In this study, we analyzed QTLs in rainbow trout, focusing on diseases such as BCWD, IHN or IPN, among others. The stability of QTLs could be influenced by different genetic backgrounds, environmental or population type. To address this variability, meta-QTL could identify consistent stable and robust QTLs by integrating data from multiple studies (Kaur et al., 2023). To the best of our knowledge, this is the first meta-analysis of QTLs related to pathogen resistance in *O. mykiss*, revealing chromosome 8 as a key genomic hotspot for pathogen resistance QTLs and consolidating finding across diverse studies. Further validation of candidate genes through GWAS is essential to facilitate practical applications in aquaculture.

**Conclusions**

This meta-analysis underscores the value of integrating data from multiple studies to identify genomic regions associated with pathogen resistance in rainbow trout. The consolidation of 26 MQTLs provides a robust foundation for developing more precise and efficient selective breeding strategies in aquaculture. The identification of chromosome 8 as a genomic hotspot for disease resistance opens new avenues for functional genomics research and supports the development of disease-resistant rainbow trout, contributing to a more sustainable aquaculture industry.

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Kaur, S., *et al.,* 2023. QTL Meta-Analysis: An Approach to Detect Robust and Precise QTL. Trop. Plant Biol., 16: 225–243.

# COMPARATIVE IN VITRO TRANSCRIPTOMIC PROFILING OF IMMUNE RESPONSE TO POLY I:C IN MARINE FISH

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## Introduction

Aquaculture is crucial to address the growing global demand for marine products, particularly in Europe, where species such as *Scophthalmus maximus* (turbot)*, Sparus aurata* (gilthead seabream) and *Dicentrarchus labrax* (seabass) are valued for their production efficiency (FAO, 2024). However, the rapid expansion of the industry has led to increased viral diseases (Lafferty *et al.,* 2015). Understanding the immune mechanisms underlying resistance to these pathogens is essential for improving aquaculture sustainability (Sood *et al.,* 2021). High-throughput transcriptomic analyses, such as RNA-seq, have emerged as powerful tools to unravel the molecular basis of immune responses in fish (Song *et al.,* 2022). *In vitro* stimulations with viral mimics like Poly I:C enables direct comparison of antiviral responses across species (Rao *et al.,* 2023). Despite these advances, comparative studies evaluating *in vitro* immune responses to viral challenges across species remain scarce, limiting our knowledge of conserved and divergent immune pathways. The aim of this study is to comparatively analyze the *in vitro* immune responses of turbot, seabream and seabass leukocytes to Poly I:C in order to identify conserved and species-specific immune mechanisms that could inform future strategies for disease resistance in aquaculture.

## Material and Methods

Leukocytes were isolated from head kidney of turbot, seabream and sea bass, pooled, and treated with Poly I:C or PBS. After 24 h incubation, RNA was extracted, quality-checked, and sequenced (Illumina NovaSeq S4, 150 bp paired-end). Reads were quality-assessed (FastQC), trimmed (FastP) and pseudo-aligned to species-specific reference transcriptomes (Kallisto). Differential expression analysis was performed with DESeq2 (FDR <0.05) and PCA for quality control. Functional enrichment (G:Profiler), GO visualization (ReViGO), and immune orthologue mapping (Ensembl Biomart) enabled comparative analysis across species, summarized by Venn diagrams and heatmaps.

## Results

A total of 36 RNA-seq datasets were generated in this study. Differential expression analysis identified a total of 1,383 DEGs in turbot, 4,335 DEGs in seabream and 504 DEGs in seabass (Fig 1). GO analysis of DEGs in the three teleost species revealed that *D. labrax* exhibited the highest number of clusters and GO terms associated with the immune response. Notably, all three species exhibit a number of common GO terms related to the immune response, response to external stimuli/pathogens and biological interactions.

Homology analysis of immune-related DEGs identified 502 homologous genes following *in vitro* Poly I:C stimulation, with 31 shared among all three species. Turbot showed higher expression of interferon-related and immune regulatory genes, while seabream and seabass exhibited increased expression of proteasome, antigen processing, and other immune system components-related genes.

## Discussion

In the current work, we examined the impact of Poly I:C, as a mimic for RNA viral infections, on transcript levels in diverse fish species using *in vitro* approaches, as a first step towards elucidating their role in the conserved defence mechanisms in teleosts. The RNA-seq results revealed a proportional overall expression response, with a constant percentage of up- and downregulated DEGs across species, although seabass exhibited fewer DEGs compared to the other species.




Fig. 1 Number (A) and Proportion (B) of Up- and Down-regulated DEGs in response to Poly I:C stimulation in *S. maximus, S. aurata* and *D. labrax*.

To identify genes potentially subject to evolutionary conservation, homologous DEGs across the three species were compared. The general observations suggested a total of 6.17% shared for the three species when a potent stimulant such as Poly I:C was employed. This results are in accordance with previous findings in salmonid fishes, where the overlap of orthologous DEGs between species is also low, likely due to virus-induced evolutionary pressures driving rapid adaptation of immune regulatory sequences (Clark *et al.,* 2023).

Noteworthy, a conserved pattern was observed across all three species, where a subset of DEGs displaying a more similar expression profile between seabass and seabream than with turbot. This is expected given the closer evolutionary relationship between seabass and seabream (Hughes *et al.,* 2018). Moreover, the shared genes were found to be mainly related to key pattern recognition receptors.

**Conclusion**

This study presents a transcriptomic analysis of the immune response to Poly I:C, a mimic for RNA virus infection, in turbot, seabream and seabass, revealing robust and conserved regulation of immune-related genes across these species. Several orthologous immune-defense genes were identified as part of shared antiviral response. These findings provide valuable molecular resources for future research on fish immunity.

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# A PILOT STUDY ON THE MOLECULAR RESPONSES OF *Seriola dumerili* TO MONOGENEAN PARASITES: A PROTEOMIC APPROACH

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## Introduction

The culture of the greater amberjack (*Seriola dumerili*) is limited by parasitic diseases, with the monogenean *Neobenedenia* spp. and *Zeuxapta seriolae* being two of the main challenges (Rigos et al., 2020). Understanding the host's molecular defense mechanisms is key to developing effective control strategies. Plasma proteomic analysis could help identify resistance biomarkers and improve management and control strategies. This study aimed to analyze and differentiate the plasma proteomic profile of *S. dumerili* in response to parasitism by both pathogens. To this end, label-free quantification (LFQ) proteomics was applied to plasma samples from fish with different parasitic loads.

## Material and methods

Label-free quantification (LFQ) proteomics was applied to plasma samples from fish with different parasite loads. Specimens of *Seriola dumerili* with varying levels of *Neobenedenia* spp. (N) and *Zeuxapta seriolae* (Z) infestation were obtained from the Canary Islands Oceanographic Centre (IEO, Tenerife). Eleven fish of similar size and weight (85 ± 9.42 cm; 11.91 ± 3.07 kg) were assigned to high (H) or low (L) infestation groups based on thresholds arbitrarily established from COC-IEO experience on the physiological and pathological effects of each parasite (>2000 *Neobenedenia*, >50 *Zeuxapta*). Four experimental categories were defined according to the H/L status for N and Z, respectively: HH (high infestation with both parasites), HL (high *Neobenedenia*; low *Zeuxapta*), LH (low *Neobenedenia*; high *Zeuxapta*), and LL (low infestation with both ≡ control), with 2–3 biological replicates each. Blood was collected via caudal venipuncture after anaesthesia with butanol chloride (30 ml/100 L) using heparinized syringes, centrifuged (2500 g, 15 min, 4 °C) to obtain plasma, stored at −80 °C, and transported to PROMEC (NTNU, Norway) for proteomic analysis. Samples were treated with lysis buffer (SDC, Tris-HCl, TCEP, CAA), heated (95 °C, 20 min), digested with trypsin (overnight, 37 °C), purified on C18 columns, dried, and resuspended in 0.1% formic acid for LC-MS/MS on a timsTOF Pro coupled to a nanoElute HPLC, using a 75 min gradient (0–40% acetonitrile). Data processing in R (v4.4.2) involved contaminant/decoy removal, variance-stabilizing normalization, and MNAR value imputation. Multivariate analyses included PCA (top 500 variable proteins), ANOVA with Tukey's post hoc for the PC, and differential expression analysis (DEP). Functionally relevant proteins were examined for GO term and pathway enrichment using g-Profiler.

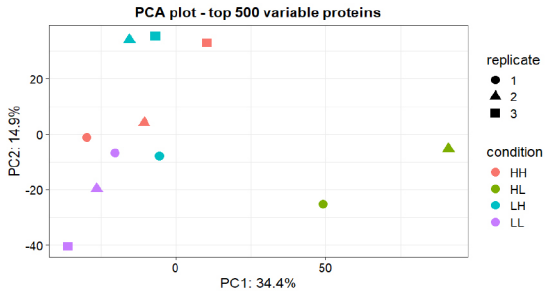


**Figure 1.** PCA of *S. dumerili* plasma samples based on the 500 most variable proteins, showing the clustering of replicates (shape) according to experimental conditions (color). The first two principal components explain 34.4% (PC1) and 14.9% (PC2) of the total variance, respectively.

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**Results and Discussion**

PCA revealed clear separation among infection conditions. HL (high *Neobenedenia*, low *Zeuxapta*) separated strongly on PC1, indicating a distinct host proteomic response. HH and LH (involving *Zeuxapta*) clustered closer to LL (low infection), suggesting a milder proteomic effect. PC2 partially separated HH and LH from LL, reflecting shared features linked to *Zeuxapta*. ANOVA on PC1 confirmed significant differences between conditions ($F_{(3,7)} = 15.82$, $p = 0.00168$), with HL showing significantly higher PC1 scores than all others. PC2 differences were not statistically significant ($F_{(3, 7)} = 3.217$, $p = 0.0919$) but suggested a marginal trend consistent with visual separation.

Proteins contributing most to PC1 and PC2 were grouped into three subsets: HNP (high PC1 loadings, linked to *Neobenedenia*), HZP (high PC2 loadings, linked to *Zeuxapta*), and LZP (low PC2 loadings). HNP proteins were enriched in proton transport and phagosome-related processes. HZP proteins related to adaptive immunity, branched-chain fatty acid metabolism, and extracellular matrix remodelling, while LZP proteins were associated with primary metabolism and protein translation.

Differential expression analysis showed no significant differences between parasitized groups and LL but revealed 11 significant proteins between HL and LH (padj < 0.05). LH displayed higher levels of proteins linked to muscle contraction, oxidative metabolism, and epigenetic regulation, whereas HL showed increased proteins related to vesicular trafficking and intracellular signalling. The lack of univariate differences with LL likely reflects its low but non-zero parasite load, while multivariate analyses (PCA) captured coordinated proteomic shifts.

The results revealed unique and specific molecular responses for each parasite. The integration of multivariate (PCA) and univariate analyses proved crucial. While few individual proteins showed significant differential expression, PCA revealed clear, distinct host response patterns for each parasite. This indicates the host's reaction is based on coordinated, systemic changes rather than the overexpression of a few key proteins.

The response to the ectoparasite *Neobenedenia* was characterized by a localized innate immune activation centered on phagocytosis, suggesting a controlled strategy to minimize systemic inflammation. In contrast, *Zeuxapta* triggered a more complex and systemic response, involving the activation of adaptive immunity (immunoglobulins, complement), indicators of tissue damage, oxidative stress, metabolic reprogramming, and epigenetic regulation. Based on our results, evasion strategies to survive within the host cannot be ruled out.

Due to the pilot nature of this study, we cannot yet define reliable biomarkers to separate infected fish from healthy ones. However, a key finding of this study, it is of a specific group of proteins can effectively tell us which of the two parasites is responsible for an infection.

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# REFINEMENT OF PROTOCOL FOR RNA EXTRACTION FROM PLASMA-DERIVED EXTRACELLULAR VESICLES IN *Spatrus aurata* FOR LNCRNA ANALYSIS

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**Introduction**

Extracellular vesicles (EVs) are membrane-bound structures that carry proteins, lipids, and nucleic acids, including mRNAs, miRNAs, and lncRNAs, enabling intercellular communication and gene regulation (Li et al., 2019). They are classified as exosomes, ectosomes, or apoptotic bodies, differing in size and origin. EVs may also mediate the transfer of epigenetic information between somatic and germline cells, potentially influencing inheritance (Spadafora, 2020).

RNA from EVs can be isolated via ultracentrifugation, size-exclusion chromatography, immunoaffinity, or commercial kits. The exoRNeasy™ kit allows simultaneous EV isolation and RNA purification with high purity, reproducibility, minimal degradation, and compatibility with small sample volumes, making it suitable for studies with limited material ((Lobb et al., 2015). While EV research is established in humans and model organisms, studies in teleost fish are emerging, showing EVs in plasma, mucus, and cell cultures with roles in immunity, stress response, and development (Lange et al., 2019; Sun et al., 2017). Standardized protocols are lacking, and preanalytical variables can affect RNA yield and integrity. Optimizing EV-RNA isolation and purification in non-model species is therefore essential for reproducible and reliable studies, which is the main goal of this work. Here we aim to optimize the protocol for RNA extraction from EVs in *Sparus aurata* using the exoRNeasy® kit, evaluating the impact of sample volume and freezing on RNA quality, and validating the presence of lncRNAs for epigenetic studies in aquaculture.

**Material and methods**

Blood from 13 fish of similar size and weight (35,42 ± 1,48 cm; 1,08 ± 0,13 kg) was collected via caudal venipuncture after anaesthesia with butanol chloride (30 ml/100 L) using EDTA-prepared syringe syringes, centrifuged (2500 g, 15 min, 4 °C) to obtain plasma. Plasma was then filtered through 0.8 μm Sartorius Minisart™ NML syringe filters to remove cellular debris and large particles and was either processed immediately after extraction (fresh) or processed after being frozen (-20°C) for two weeks. EVs were isolated from plasma and their RNA purified using the commercial exoRNeasy Kit (QIAGEN, 2019) following the manufacturer's instructions with minor adaptations. Both exoRNeasy Midi and Maxi kits were used, with the Midi kit applied for treatment comparisons (fresh vs -20°C) and the Maxi kit only for sample volume comparisons. the quality of the EVs was assessed by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern) and transmission electron microscopy (TEM). RNA concentration and integrity were quantified with Qubit® using RNA HS Assay and TapeStation®, respectively. Nanopore sequencing (MinION) was performed to characterize the lncRNAs. cDNA libraries were prepared with the PCR-cDNA Barcoding Kit 24 (SQK-PCB114-24, ONT). Sequencing reads were aligned to the gilthead sea bream reference genome (fSpaAur1.1, RefSeq 2019) via EPI2ME, normalized by rarefaction, and quantified using GenomicAlignments in R with strict intersection and multimapping assigned to all overlapping genes to enhance sensitivity for low-abundance lncRNAs. Transcript size distributions, RNA biotype proportions, and the lncRNA/mRNA ratio were evaluated.

Statistical analyses were performed in R, using parametric (t-test) or nonparametric tests (Mann–Whitney, permutation, bootstrap) depending on data distribution, and transcript size distributions were compared with Kolmogorov–Smirnov and $\chi^2$ tests.

**Results and Discussion**

Validation of the exoRNeasy kit in gilthead sea bream: The exoRNeasy kit efficiently isolated EVs and purified total RNA, including lncRNAs, from *Sparus aurata* plasma, establishing a reproducible protocol for non-model aquaculture species and representing the first validation of this kit in this species.

Sample volume and yield: No significant differences in RNA yield were observed between the Midi (100–1000 μL) and Maxi (1–4 mL) kit formats, allowing flexibility in sample volume without compromising efficiency.

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Impact of preprocessing: Although no statistically significant differences were detected, freezing tended to reduce RNA concentration (p=0.01). Further studies with larger sample sizes are needed to confirm these trends. Freezing, significantly affected transcript size distribution transcripts. $\chi^2$ tests using 100 bp and 250 bp intervals revealed highly significant differences across all three subsamples ($p < 0.001$). reducing long transcripts (>800 bp). Results were consistent regardless of interval size, indicating robust transcript size distribution differences.

These findings support the impact of preprocessing on long transcript representation in EV RNA.

EV characterization: Isolated EVs showed variable sizes and typical morphology, with RNA profiles consistent with those commonly observed in EVs.

Detection of lncRNAs in EVs: The presence of lncRNAs was confirmed, identifying 302 distinct transcripts, demonstrating the suitability of the selected protocol for lncRNA detection.

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# COMPARATIVE TRANSCRIPTOMIC PROFILING OF TWO GEOGRAPHICALLY DIFFERENT *Philasterides dicentrarchi* STRAINS PATHOGENIC OF TURBOT (*Scophthalmus maximus*)

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Introduction

Scuticociliatosis is a severe protozoan disease caused by ciliates, posing a significant economic burden on the global aquaculture sector. In Spain, it was first reported in turbot *(Scophthalmus maximus)* by Iglesias et al. (2001), with *Philasterides dicentrarchi* identified as the causative agent. Although *P. dicentrarchi* is considered a facultative parasite, its adaptability, rapid reproduction, and environmental resilience enable efficient spread and colonization, leading to high mortality rates of turbot production in Galicia. It is closely related to *Miamiensis avidus*, a scuticociliate affecting.

Outbreaks are typically associated with elevated seawater temperatures, and climate change is expected to increase both prevalence and geographic range, making *P. dicentrarchi* an emerging pathogen. As developing chemotherapeutic treatments is challenging due to strict regulations and environmental concerns, preventive measures, particularly vaccination, become the most viable approach. An autologous vaccine against scuticociliatosis, developed by CETGA, provides effective protection against the specific strain involved in a given outbreak. However, there is no crossprotection between geographically distinct isolates, such as those from northern and southern Galicia. Identifying conserved antigens across *P. dicentrarchi* strains and understanding their gene regulation could pave the way for broader, more effective disease control strategies. In this study, the transcriptomes of 2 *P. dicentrarchi* isolates from CETGA´s collection, were compared.

Materials and methods

**RNA Isolation and Sequencing**: Samples obtained from *P. dicentrarchi* cultures were mechanically homogenized in a TissueLyser II (Qiagen), and RNA was extracted. RNA quality and quantity were assessed using an Agilent 2100 Bioanalyzer with the RNA 6000 Nano Kit.

**Library Preparation and Sequencing, *de novo* assembly, RNAseq, and bioinformatic analyses** were carried out by AllGenetics & Biology SL (www.allgenetics.eu)".

**Differential expression analysis:** a differential expression analysis was conducted using the Bioconductor packages NOISeq and NOISeqBIO (Tarazona et al., 2013), which allow for the use of data obtained from biological replicates. The probability threshold was set to q = 0. For the differential analysis, only proteins with <1.5 fold change, were considered. Functional analysis with STRING was performed. *Tetrahymena thermophila* database was used as a model to annotate transcripts from *P. dicentrarchi* in STRING: functional protein association networks (https://string- db.org/) (Szklarczyk et al., 2023). STRING was used for searching protein interaction evidence.

STRING settings were based on evidence, with the high confidence (0.700) for interaction score. A minimum number of nodes was added when needed to reach a significant PPI enrichment p-value. Proteins were then grouped into clusters in STRING using the k-means clustering method. Functional analysis was performed including biological process, molecular function and cellular component. Functional analysis was performed separately for upregulated and downregulated genes.

Results

The differential expression analysis of the comparison "Control P6 vs P9" identified a total of 5532 differentially expressed features. Of these, 2827 features were up-regulated and 2705 features were down-regulated in the control P6 group.

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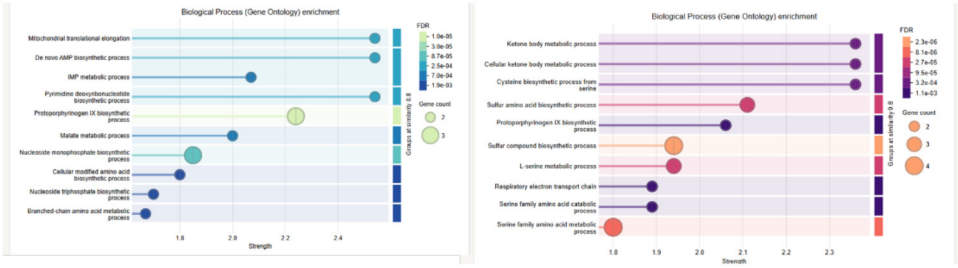


Figure 1: Most representative Biological processes represented in the networks formed by differentially expressed genes (DEGs), for significativelly upregulated (left) and downregulated (right).

Regarding functional analysis with STRING, for the upregulated genes, k-means clustering identified six main functional groups. The most enriched biological processes, with a strength greater than 2.0, included mitochondrial translational elongation, de novo AMP biosynthesis, pyrimidine deoxyribonucleotide biosynthetic process, protoporphyrinogen IX biosynthetic process, IMP metabolic process, and malate metabolic process. Concerning the downregulated genes, the main biological processes involved ketone body metabolism, cysteine biosynthesis, and sulfur amino acid metabolism. In terms of molecular functions, the most notable enrichments included oxidoreductase and oxoacid activities, ribonuclear protein complexes, and mitochondrial-associated processes.

## Conclusion

The functional clustering of upregulated genes suggests that *P. dicentrarchi* responds to infection or environmental stimuli with a marked activation of metabolic and biosynthetic pathways, particularly those involved in nucleotide synthesis, amino acid metabolism, and mitochondrial activity. The enrichment of mitochondrial translational elongation, AMP and pyrimidine biosynthesis, and TCA cycle-related components indicates an increased demand for energy production and macromolecular synthesis, consistent with a proliferative or metabolically active state in the P9 isolate, the more virulent one.

Conversely, the downregulated gene clusters point to a suppression of certain catabolic and structural processes, including branched-chain amino acid degradation, ribosomal structural protein expression, and specific molecular motor functions such as dynein activity. The reduced activity of ribonucleoprotein complexes and translational GTP-binding proteins may reflect a reallocation of cellular resources away from housekeeping functions toward processes critical for survival and adaptation.

Overall, the data reveal a metabolic reprogramming in *P. dicentrarchi*, characterized by the upregulation of biosynthetic and energy-related pathways and the downregulation of components associated with protein degradation, structural maintenance, and motility. This shift likely supports rapid growth, pathogenicity, and adaptation during infection, and highlights potential targets for therapeutic intervention.

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# EFFECT OF HALOPHYTE-DERIVED BIOACTIVE COMPOUNDS ON THE INTESTINAL HEALTH OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

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## Introduction

The use of bioactive compounds derived from macro- and microalgae in aquafeeds has shown a positive effect on the overall welfare of cultured fish, improving growth performance, digestive function, metabolism, immune status, and stress resilience. Moreover, the inclusion of these bioactive compounds has been associated with enhanced intestinal health and integrity, as evidenced by increased transepithelial electrical resistance and improved apparent epithelial permeability, measured using electrophysiological methods (Caderno *et al*., 2025). Halophyte plants, which grow in highly saline environments, have also emerged as promising sources of bioactive compounds. These species are increasingly recognized as valuable tools in sustainable aquaculture due to their potential to enhance immune response and disease resistance in European seabass (*D. labrax*) (Machado *et al*., 2024). The aim of the present study is to evaluate the effects of various halophyte-derived bioactive extracts on the growth and intestinal health of European seabass by using a preliminary *ex vivo* assessment of the organism's physiological response to each extract to select the most promising for subsequent *in vivo* experiments.

## Materials and methods

This work was structured into three trials involving different electrophysiological techniques, using voltage clamp in Ussing chambers and permeability assays.

Trial 1. An *ex vivo* assay was conducted using juvenile seabass fed a standard commercial diet. Intestinal tissue sections (Anterior Intestine - AI; Mid Intestine - MI; Posterior Intestine - PI) were isolated and mounted on tissue holders to determine the most efficient intestinal region for electrogenic amino acid transport.

Trial 2. MI sections were used for *ex vivo* electrophysiological trials, where seven different halophyte-derived bioactive extracts (FERM, FRAC3, XAD1, XAD2, XAD3, EXT2 and EXT3) were tested at a concentration of 1 mg/mL. The extract showing the most favorable response was selected for further *in vivo* testing.

Trial 3. An *in vivo* feeding trial (30 days) was carried out with juvenile seabass with four different diets: i) Control Diet, without aquafeed supplementation, or supplemented with ii) 0.25% (Diet 1), iii) 0.5% (Diet 2), and iv) 1% (Diet 3) of EXT3, in triplicate. After the feeding period, MI sections were collected from 12 individuals per treatment and electrophysiological techniques were used to evaluate intestinal health and integrity by means of tissue resistance, amino acid transport, and apparent permeability.

## Results and discussion

Preliminary *ex vivo* assays using different intestinal sections revealed that the MI exhibited significantly higher electrogenic amino acid transport compared to both AI and PI sections. As a result, the MI was selected for all subsequent analyses. This finding suggests that the MI is the primary region for nutrient absorption, consistent with previous work by Fuentes *et al*. (2025). Based on this, different halophyte-derived bioactive extracts were tested on MI sections to identify the most effective compound, where EXT3 was ultimately selected as the most promising extract for the *in vivo* feeding trial.

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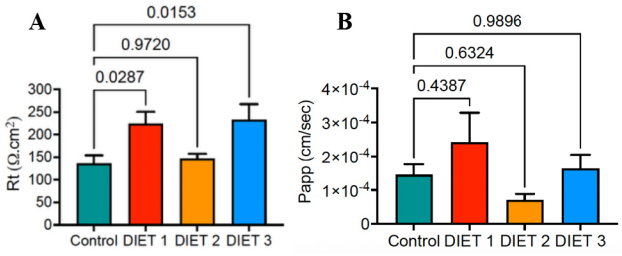


**Figure 1**. Effect of different diets: A) on intestinal tissue resistance (Rt, Ω $cm^{-2}$), B) on intestinal apparent permeability (Papp, cm/sec). Control: Commercial diet; Diet 1, 2 and 3: Experimental diets with 0.25%, 0.5% and 1% EXT3 inclusion respectively. Each column represents the average ± SEM (n = 12). Statistics among dietary treatments were based on one-way ANOVA followed by Tukey's post-hoc test, indicating significant differences between experimental groups.

After one month of feeding seabass with either a control or three experimental diets, no significant differences in growth performance were observed. However, all experimental diets enhanced amino acid transport in intestinal tissues, with the highest increase observed in DIET3 (1% EXT3). Regarding intestinal integrity, epithelial resistance (Rt) was significantly higher in DIET1 and DIET3 (Fig. 1A), which was consistent with the same (although no significantly different) pattern observed for apparent permeability (Papp, Fig. 1B). These results are indicative of a putative improvement of intestinal functioning after a short-feeding period of this tested compound.

**Conclusions**

The seabass mid intestine (MI) exhibited the highest electrogenic amino acid transport activity, confirming it as the primary site for nutrient absorption and the most suitable region for *ex vivo* electrophysiological assessments. In addition to that, among the halophyte-derived bioactive extracts tested, EXT3 demonstrated the most favorable effects on intestinal function to be used for *in vivo* validation. Even so, although no significant differences in growth performance were observed after 30 days of feeding, all diets containing EXT3 improved amino acid transport across the intestinal epithelium, with the highest and most positive effect seen in the diet containing 1% EXT3 (DIET3).

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**Acknowledgments**

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# FISH GROWTH AND WELL-BEING: THE ROLE OF GRAPE MARC NUTRACEUTICALS ON THE PHYSIOLOGY OF *Seriola dumerili*

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## Introduction

In the context of a more sustainable and environmentally friendly aquaculture, there is a growing interest in incorporating non-conventional feed ingredients that provide essential nutrients and bioactive compounds beneficial to fish health and welfare. Among these compounds, grape marc polyphenols stand out for their antioxidant, immunostimulant and anti-stress properties. These attributes contribute to enhancing the overall health status of fish, improving their resistance to pathogens, and reducing the reliance on antibiotics and chemical additives. Consequently, grape marc polyphenols represent a promising natural alternative for improving both the quality and sustainability of aquaculture production (Martínez-Antequera, 2023).

## Materials and methods

In order to evaluate the effect of Albariño grape marc (*Vitis vinifera*) extract inclusion in the diet of juvenile greater amberjack (*Seriola dumerili*), three experimental diets were formulated: a control diet, corresponding to a standard commercial formulation for this species, and two additional diets supplemented with 750 and 1500 ppm of Albariño grape marc extract (i-Grape grape extract® 500 mL, i-Grape Laboratories, Santiago de Compostela, Spain), respectively. The experiment was conducted in triplicate. During the experimental feeding period (77 days), fish were fed *ad libitum* 6 days per week, with four daily meals (9:00 am, 12:00 pm, 15:00 and 18:00 pm). In addition to the initial and final samplings, two intermediate biometric samplings were carried out during the trial. At the end of the feeding trial, four animals per tank were anaesthetised with a lethal dose of 2-phenoxyethanol and blood, liver and muscle samples were taken for further analysis. In addition, intestinal tissue samples were also taken for *ex vivo* testing using electrophysiological techniques to observe the effect of different diets on intestinal health and integrity through resistance (Rt), active net transport capacity ($I_{sc}$) and apparent tissue permeability (Papp).

## Results and discussion

Although no significant differences in growth or feed conversion ratio (FCR) were observed, fish fed the diet supplemented with 1500 ppm of grape marc extract showed a clear trend toward greater growth and reduced feed intake. It is likely that, with a longer trial period, the diet with the highest polyphenol concentration (1500 ppm) would have resulted in a significantly improved growth and feed efficiency, thus enhancing sustainability and cost-effectiveness. Moreover, the protein efficiency ratio (PER) showed a dose-dependent increase, being highest with the 1500 ppm diet, suggesting better protein utilization. In addition to that, polyphenols can influence lipid metabolism (Lizárraga & Hernández, 2018). In our study, fish fed the 750 ppm diet showed greater fat mobilization, with higher plasma triglyceride (TAG) levels (Fig. 1A). This diet also appears to reduce stress, as indicated by lower cortisol levels (Fig. 1B), likely due to its antioxidant and anti-inflammatory properties. Furthermore, hepatic cholesterol levels (Fig. 1C) decreased with increasing dietary polyphenol content, potentially aiding in the prevention of fatty liver and improve the use of fats as an energy source. These effects are attributed to the capacity of polyphenols to mitigate oxidative damage and inflammation, thereby preventing hepatic lipid accumulation and supporting a more balanced metabolism (Lizárraga-Velázquez *et al.,* 2018).

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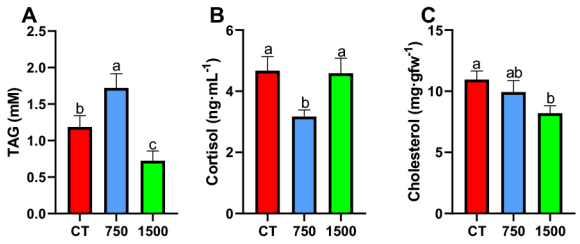


**Figure 1**. Results of plasmatic and hepatic metabolites. A) Plasma triglycerides (TAG), B) Plasma cortisol and C) Hepatic cholesterol in *S. dumerili* with different experimental diets (mean ± SEM, n=12). Different letters indicate significant differences among dietary treatments based on one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Regarding the results obtained through electrophysiological measurements in intestinal tissue, we observed that none of the diets altered the epithelial barrier function or resistance. On the other hand, both diets supplemented with polyphenols induced a modification in the active net transport capacity of the intestine. Finally, the apparent permeability values were significantly lower in the diet with 750 ppm compared to both the control diet and 1500 ppm groups, suggesting a modification in intestine configuration.

## Conclusions

Although growth performance and feed conversion were not significantly affected within the trial duration, fish fed the highest marc grape polyphenol dose (1500 ppm) showed trends toward improved growth, reduced feed intake, and significantly enhanced protein efficiency ratio and condition factor, indicating better nutrient utilization and welfare. 750 ppm diet appears to preserve a greater integrity and functionality of the intestinal epithelium, as it does not show altered resistance values but does exhibit lower apparent permeability compared to the other diets. Moreover, the 750 ppm diet led to more efficient lipid metabolism, as evidenced by higher plasma TAG, lower cortisol, and reduced hepatic cholesterol levels. These results suggest that dietary polyphenols can promote metabolic balance, reduce oxidative stress, and improve feed utilization, thus offering a promising functional strategy to enhance sustainability in aquaculture systems.

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# HARNESSING THE POWER OF PROBIOTICS: PREVENTING AND CONTROLLING INFECTIOUS DISEASES IN ATLANTIC SALMON AQUACULTURE

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Infectious diseases remain a persistent and costly challenge in Atlantic salmon (*Salmo salar*) aquaculture, contributing to mortality rates, compromised animal welfare, and economic losses. Central to the first line of defense of the host are mucosal surfaces which are the gills, skin, and gut, serving as a critical immunological interface with the external environment. These barriers are not only sites of pathogen entry but also hubs of complex host-microbiota-pathogen interactions that can determine infection outcomes. Consequently, strategies that modulate mucosal microbiota, such as probiotic administration, have gained growing attention. This study critically examined the current body of evidence on the microbiota composition in healthy salmon and modulation via the application of probiotics to reinforce mucosal barrier function and microbiome resilience in salmon. Probiotic strains, including *Pediococcus acidilactici*, *Carnobacterium* spp., and *Lactococcus lactis*, have demonstrated protective effects through competitive exclusion, enhancement of innate immunity, and reduction of pathogen load in challenge experiments. However, findings across studies often diverge due to strain-dependent effects and lack of consistency in experimental designs, highlighting a need for more harmonised methodologies. A significant bottleneck in the field is the limited and inconsistent use of microbial profiling tools such as 16S rRNA gene amplicon sequencing, making cross-study comparisons difficult and potentially hindering progress toward evidence-based probiotic application. Despite promising results, significant knowledge gaps remain regarding the mechanisms of action, host-microbe interactions, long-term impacts, and the lack of standardised protocols for probiotic research in salmon. Addressing these gaps will be essential for developing robust, evidence-based probiotic interventions for disease management in Atlantic salmon farming.

# ROLE OF THE INTESTINAL MICROBIOTA IN WEIGHT AND PRODUCTIVE PERFORMANCE OF RAINBOW TROUT (*Oncorhynchus mykiss)*

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## Introduction

The intestinal microbiota constitutes a central regulator of host metabolism, influencing nutrient digestion, energy balance, and lipid deposition. Several studies in poultry, pigs, and fish have demonstrated that changes in microbial composition or activity impact growth performance, with short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate acting as additional energy sources and modulators of lipid metabolism (Yao et al., 2024; Shi et al., 2025). These microbial functions enhance nutrient utilization and contribute directly to weight gain, a parameter of high commercial importance in animal production systems.

Despite these advances, most research has focused on indirect modulation of the microbiota through diet, probiotics, or environmental conditions, rather than by direct disruption. To address this gap, we applied a strategy based on the administration of non-absorbable antibiotics (neomycin and bacitracin) to selectively disturb the intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). This approach enabled us to evaluate more specifically the role of the microbiota in host growth and productive performance, providing insights into the metabolic contribution of intestinal bacteria in aquaculture species.

## Materials and Methods

The trial was conducted at the Aquaculture Center, Faculty of Agronomy, University of Chile, using rainbow trout (*Oncorhynchus mykiss*) with an initial mean weight of ~70 g. After two weeks of acclimation in 400 L tanks within a recirculating aquaculture system (RAS, 15°C), 340 fish were distributed into four experimental conditions (Neomycin, Bacitracin, Neomycin+Bacitracin, Control) with two replicates per group (40 fish/tank). Fish were fed a commercial diet at 1.5% BW throughout the trial. Antibiotics were administered via force feeding at 100 mg/kg BW, a dose previously validated to reduce cultivable microbiota without affecting biofilter performance. Sampling was carried out at days 3, 30, and 60 post-treatment, with 14 fish per condition anesthetized, weighed, measured, and blood-sampled prior to euthanasia. Tissues collected included viscera, liver, muscle, and intestinal content/mucosa, which were stored at –20°C or –80°C depending on downstream analysis.

Biometric indices (VSI, HSI, CF) and productive parameters (WG, SGR, FCR) were calculated using standard formulas from individual and tank-level data. Statistical analyses were performed in RStudio; normality and homogeneity were tested (Shapiro–Wilk, Levene), and pairwise comparisons with the control were evaluated using t-test, Welch, or Wilcoxon tests as appropriate. Figures were generated with the ggplot2 package.

## Results

At 60 days post-treatment, fish in the NB group showed significantly lower body weight and carcass weight compared to the control, while groups N and B did not differ from C. Body length was also greater in the control group than in all antibiotic-treated groups at this time point. No significant differences were detected in liver or viscera weight across treatments. Regarding productive indices, feed conversion ratio (FCR) did not differ among groups, whereas the specific growth rate (SGR) was significantly reduced in NB compared to the control. Importantly, feed intake remained similar across treatments, suggesting that the lower growth observed in NB fish was not related to reduced consumption but rather to alterations in nutrient utilization efficiency.

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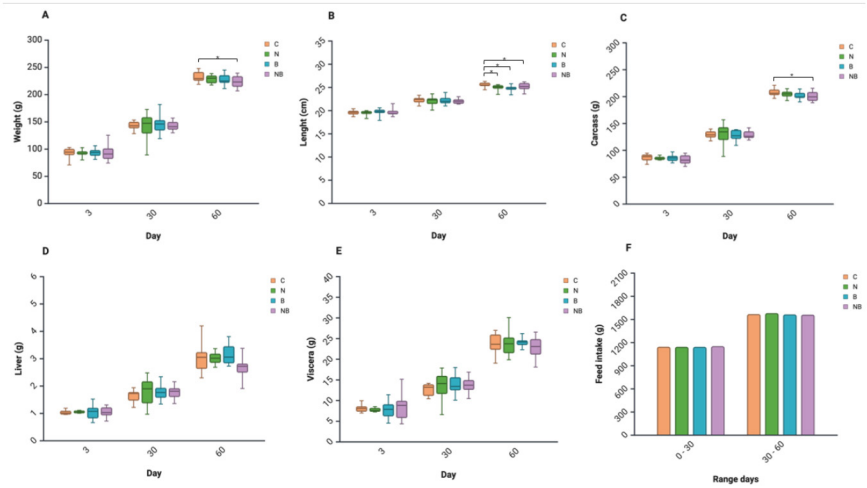


**Discussion**

The combined treatment with neomycin and bacitracin (NB) significantly reduced body weight, carcass weight, and specific growth rate (SGR) compared to the control, while individual antibiotic treatments (N, B) did not show these effects. This suggests that the simultaneous targeting of Gram-negative and Gram-positive bacteria caused a broader disruption of the intestinal microbiota, impairing microbial functions linked to nutrient utilization and energy metabolism. In mammals, Nemoto et al. (2023) reported that germ-free mice showed lower body weight than conventional counterparts despite equal food intake, largely due to reduced lipid deposition. A similar mechanism may explain our findings, as disruption of the microbiota in trout could compromise lipid metabolism, limiting energy storage and consequently growth performance.

The decrease in carcass weight indicates that the negative impact of NB was expressed mainly as reduced somatic growth rather than changes in visceral or hepatic mass, since liver and viscera weights did not differ among treatments. Thus, the observed reduction in growth and SGR may reflect not only diminished nutrient absorption efficiency but also an altered balance of lipid metabolism, resulting in lower deposition of energy reserves in muscle tissue. Together, these results reinforce the role of the intestinal microbiota in supporting both energy harvest and lipid storage, processes that are critical for productive performance in aquaculture species.

# NUCLEAR ESTROGEN RECEPTORS OF EUROPEAN SEA BASS (DICENTRARCHUS LABRAX) AS TARGETS OF ENDOCRINE DISRUPTING COMPOUNDS: EFFECTS ON REPRODUCTION AND RISK ASSESSMENT POTENTIAL

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**Introduction**

Estrogens are involved in the regulation of a wide range of processes in teleost reproduction (Lubzens et al, 2010; Schulz et al, 2010) and exert their functions mainly through ligand-activation of their specific cognate receptors. Two main classes of estrogen receptors have been reported in vertebrates: membrane-associated estrogen receptors (GPER), which are G-protein coupled receptors that mediate rapid nongenomic actions through intracellular signaling pathways (Filardo et al, 2012), and nuclear estrogen receptors (ESR), transcription factors that bind to estrogen response elements (EREs) on gene promoters to regulate their expression. (Tsai et al, 1994). Three Esr subtypes have been identified in most teleost species, Esr1, Esr2a (also named Esr***β***1 or Esr***β***a), and Esr2b (also named Esr***β***2 or Esr***β***b), of which Esr2a and Esr2b coding genes arose from the whole genome duplication event specific of the teleost lineage. Differences in binding affinity and seasonal expression patterns in reproductive tissues among estrogen receptor subtypes suggest different roles during gametogenesis along the brain-pituitary-gonad axis. Endocrine-disrupting compounds (EDCs) are known to act as agonists or antagonists of steroid receptor functions and interfere in the production of the receptors themselves. Thus, many different points in the endocrine control of fish reproduction can be potential targets for the actions of EDCs. In aquaculture, the presence of estrogenic compounds is a concern, both in water and in newly formulated commercial fish diets (Arpin-Pont et al, 2016; Nacher-Mestre et al, 2013;). This study aimed to investigate the role of the three nuclear estrogen receptor subtypes in European sea bass, assess the impact of endocrine-disrupting compounds on nuclear estrogen receptors using transactivation assays, generate *in vitro* stable clones, and evaluate their potential as bioassays tools for risk assessment.

**Material and Methods**

To investigate the role of the three nuclear estrogen receptors subtypes in European sea bass, analysis of the expression of their coding genes during a whole reproductive cycle was investigated by qPCR in testis, ovary and pituitary. Localization of the nuclear estrogen receptors along the brain-pituitary-gonad axis was performed by immunohistochemistry using specific antibodies for each subtype.

Using transactivation of an ERE-luciferase reporter gene assay, transient transfection was conducted in human embryonic kidney cell line (HEK293), to functionally characterize the three sea bass nuclear estrogen receptors. The effect of two possible EDCs, genistein – a phytoestrogen also present in fish meals – and fluoxetine – an antidepressant, mainly constituent of Prozac- were assessed across all Esr subtypes.

Based on results from transient transactivation assays, stable HEK293 clones expressing each Esr subtype were generated using antibiotic selection with G418 and Hygromycin. Clones were screened by treatments with $10^{-8}$ M 17β-estradiol ($E_2$), using ethanol as vehicle control. The most responsive clones were expanded and cryopreserved. Subsequent transactivation assays using $E_2$ concentrations ranging from $10^{-5}$ M to $10^{-10}$ M were performed to determine the $EC_{50}$ (half-maximal effective concentration) and $E_{max}$ (the maximum response) values for each sea bass Esr subtype. Finally, to evaluate the potential of these stable clones as *in vitro* bioassay tools for EDCs risk assessment, environmental and biologically relevant samples (water, fish feed) were tested.

**Results**

The coding genes of the three nuclear estrogen receptor subtypes of sea bass are highly expressed in reproductive-related tissues such as pituitary and gonad. Quantitative analysis of *esr1*, *esr2a* and *esr2b* expression in the gonad and pituitary across the reproductive cycle revealed subtype and stage-specific expression patterns. Immunohistochemical localization along the pituitary-gonad axis confirmed differences among receptor subtypes depending on the gonadal stage.

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Functional characterization using an ERE-luciferase reporter assay demonstrated distinct ligand affinities among the Esr subtypes, which translated into differential transactivation responses to the EDCs genistein and fluoxetine.

Furthermore, stable HEK293 clones expressing each sea bass Esr subtype were successfully generated and validated using dose-responses assays with $E_2$ as natural ligand. Values for EC50 and Emax align with previous activations in transient transfections. Finally, the effect of different environmental and biological relevant samples on Esr subtypes confirm the reliability of these stable cell lines as *in vitro* bioassay tools for detecting and evaluating the activity of potential EDCs.

**Conclusion**

These findings indicate that the three nuclear estrogen receptors in European sea bass have non-redundant, subtype-specific roles in the regulation of gametogenesis. Consequently, exposure to EDCs—whether present in water or newly commercial fish feeds—may disrupt reproductive processes. Moreover, the development of stable Esr-expressing HEK293 clones offers a potential *in vitro* bioassay platform for the detection and risk assessment of EDCs in environmental and biological samples, with particular relevance to marine aquaculture.

**Acknowledgements**

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# COMPARATIVE BIOFOULING ANALYSIS OF COPPER-ALLOY AND NYLON NETTING FOR OFFSHORE AQUACULTURE INTEGRATED WITH MARINE RENEWABLE ENERGY

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The integration of offshore aquaculture with marine renewable energy platforms requires structural materials that minimize biofouling to ensure water exchange, reduce maintenance costs, and safeguard fish welfare. This study evaluated the colonization dynamics of marine biota on copper-alloy netting and conventional nylon netting, deployed simultaneously under identical environmental conditions at the Port of Taliarte (Gran Canaria, Spain).

Net panels (50×50 cm; n=3 per material and sampling) were mounted alternately on a submerged frame at 1.5–3 m depth, south-facing, and monitored over 89 days. Sampling at days 0, 28, 57, and 89 included wet and dry biofouling biomass, taxonomic identification, and percentage cover analysis using ImageJ-Fiji. Environmental parameters (temperature, dissolved oxygen) were recorded throughout.

Copper nets showed no detectable biofouling at day 28, while nylon nets reached 4.5 g dry weight (0.0018 g/cm²). At day 89, mean biofouling biomass was 12.38 g (0.0050 g/cm²) on copper and 15.57 g (0.0062 g/cm²) on nylon. Maximum surface coverage reached 1.20% at day 89 on copper versus 4.74% on nylon. *Cladophoropsis membranacea* was the dominant alga on both materials, but *Ellisolandia elongata* and *Neogoniolithon orotavicum* showed a clear preference for nylon. Amphipods, particularly *Ericthonius punctatus*, dominated the faunal assemblage, with sporadic decapods (*Natantia*) detected on nylon.

Temperature increases coincided with higher biomass growth, while oxygen effects were non-linear, peaking at ~7.8 mL/L. Copper nets maintained lower colonization rates and mesh permeability for longer periods, suggesting reduced cleaning frequency, lower operational costs, and decreased pathogen risk.

The results confirm that copper-alloy netting effectively delays early biofouling settlement compared to nylon under the same offshore conditions, supporting its potential application in integrated aquaculture–renewable energy platforms. Multi-season trials are recommended to assess long-term durability, seasonal variability, and economic feasibility at commercial scale.

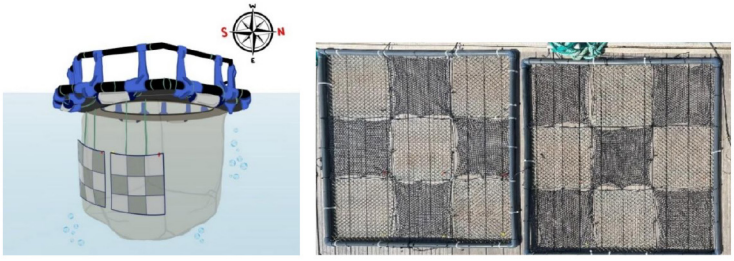

*Illustration 1. Location in prototype cage and design of the sampling quadrants.*

# PERFORMANCE AND HEALTH ASSESSMENT OF AN OFFSHORE RENEWABLE-INTEGRATED CAGE PROTOTYPE: COMPARATIVE TRIALS WITH SPARUS AURATA

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Offshore aquaculture is expanding through technologies that enable large-scale, sustainable fish farming. Within the *AquaWind* project, we compared a prototype cage, designed for integration with offshore renewable energy platforms, with a conventional commercial cage. The prototype featured a copper alloy net and remote monitoring systems, and was tested with gilthead seabream (*Sparus aurata*) in two consecutive trials.
In the first 42-day trial, fish were juveniles with an initial mean weight of 33.60 ± 12.51 g. Survival was 83.7% in the prototype versus 94.0% in the control, with lower specific growth rate (SGR 1.42% day$^{-1}$ vs. 2.04% day$^{-1}$) and reduced lipid reserves. These results were linked to operational noise, suboptimal manual feed supply, and a *lymphocystis* disease (LCD) outbreak confirmed histologically, which impaired condition and increased plasma stress markers.

The second 64-day trial (7 March to 9 May) involved adults in the final grow-out phase (initial mean weight 557.10 ± 110.50 g). Survival exceeded 96% in both cages, with no significant differences in growth (SGR ≈ 0.30% day$^{-1}$) or biochemical parameters. The lower SGR reflects the limited growth potential at this production stage. Achieving equivalent production performance in both cages demonstrates that the optimized feeding and husbandry —enabled by an remoted controlled and automated feeding system in the prototype cage to replicate commercial practices— can overcome earlier performance gaps. The copper alloy net effectively reduced biofouling, lowering maintenance needs.

These findings confirm the feasibility of automated, remotely operated offshore aquaculture to be integrated with renewable energy prototype, and highlight the importance of cage design and feeding strategies to ensure fish welfare and production efficiency across different life stages.



*llustrations. Prototype cage and operation of remote feeding system.*

# ACUTE PHYSIOLOGICAL RESPONSES OF RAINBOW TROUT TO THREE ELECTROFISHING DEVICES: A LABORATORY STUDY

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Electrofishing has been used widely as a standardized sampling technique for assessing density of fish. Although primarily used in fisheries and ecological studies, it could also play important roles in aquaculture practices such as for wild broodstock capture or the monitoring of stocked fish populations. However, the complex and dynamic impact of electricity on fish health and welfare still remain poorly understood.

In this laboratory-study, we investigated the effect of three electrofishing devices: LR-20 (Smith-Root), L-600 (Lug AB) and FA-50 (Terik Technology) on the acute stress response of rainbow trout (*Onchorynchus mykiss*). Forty to forty-six fish with an initial weight of 220 g were distributed into four circular 400 L tanks. Three tanks were exposed to the same protocol, 10 sec electrical pulse, followed by 5 sec of rest and then a second 10 sec period of electrical pulse. Ten fish from each treatment were sampled for blood and plasma at 0, 1, 6 and 24 h post exposure. A fourth tank served as undisturbed control, sampled at the same time points. After 6 h, fish showing difficulties swimming and balance issues were euthanized.

Our results show that the different electrofishing devices impacted the primary and secondary stress response differently. Overall, the LR-20 seemed to induce the lowest acute stress response. Two fish died and one fish was euthanized after 6 h in this group while six fish were euthanized with the L-600. No fish survived past 6 h with the FA-50, with 11 dead and four fish euthanized. The cortisol peaked between 1 and 6 h in all the experimental groups, with the LR-20 displaying the lowest levels (ca. 45 ng/mL) at both time points, while the FA-50 peaked at over 100 ng/mL. Both LR-20 and L-600 cortisol levels were back to basal levels after 24 h (Fig 1a). Glucose levels showed a similar pattern (Fig 1b), with an initial increase already after 1h for the L-600 and the FA-50, while the RT-20 was only significantly higher than the control after 6 h. There was no difference between the RT-20 and the L-600 for this parameter. Plasma osmolality, blood hemoglobin, hematocrit, and MCHC also indicated a mild transient stress effect, most pronounced in fish exposed to the FA-50. These more severe effects may be related to the type of electrical current used by the FA-50 (AC) known to cause more extensive neuromuscular disruption compared to pulsed DC used by the LR-20 and L-600.

Further studies are needed to assess potential long-term and indirect effects, including impacts on growth, skin integrity, and pigmentation. Additional field-based comparisons are also warranted in order to validate the results generated from different electrofishing gears.

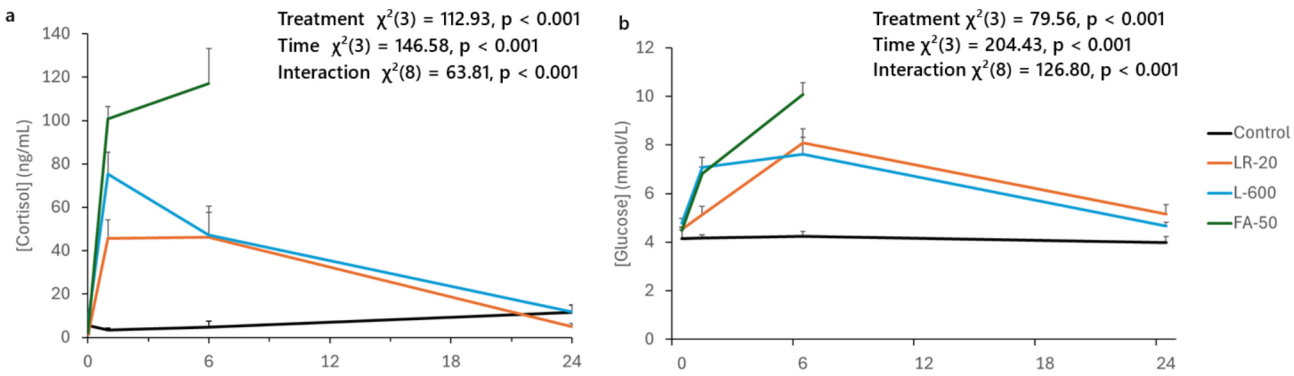


**Figure 1.** Means (± SEM) of the plasma cortisol (a) and glucose (b) of rainbow trout 0, 1, 6 and 24 h after electrofishing. Different letters indicate statistically significant differences between treatments (GLM, Wald Chi-square tests).

# ADVANCING CELLULAR AQUACULTURE BY ESTABLISHING MEAGRE *Argyrosomus regius* CELL LINES AND DEVELOPING A CIRCULAR BIOPROCESS USING MICROALGAE

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## Introduction

The global collapse of some fisheries and ongoing challenges in aquaculture highlight the urgent need for alternative sources of marine animal proteins. Cellular aquaculture - the production of these proteins from cells rather than whole animals - offers a sustainable and ethical solution to mitigate seafood shortages by reducing natural resource use and environmental impact. However, key challenges remain, particularly the development of robust marine animal cell lines, the formulation of animal-free culture media, and the reduction/upcycling of waste effluents through circular cultivation systems.

## Results

To address the scarcity of marine animal cell lines, we established and characterized lines from the meagre *Argyrosomus regius*, a commercially valuable species with limited aquaculture production. Cells were obtained from explant cultures of vertebra (VAR3), branchial arch (BAAR1) and jaw (JAR5) tissues using established protocols and propagated in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum (FBS). Cultures were maintained for over 100 passages. Growth of spontaneously immortalized lines (>60 passages) was optimized by reducing serum concentrations and adjusting incubation temperatures. Growth curves, population doubling times (PDT), biomass yield, and differentiation potential into myogenic and adipogenic lineages were evaluated, and preliminary results indicate reduced FBS requirements without compromising growth, enhanced proliferation at 33°C, with observed PDTs of 11.1 ± 0.5 h for VAR3, 25.2 ± 0.5 h for BAAR1, and 19.9 ± 0.7 h for JAR5, and successful lipid accumulation throughout adipogenic differentiation (Figure 1).

To enhance the sustainability of cellular aquaculture, we developed a circular bioprocess that integrates microalgae into the cell culture system. This approach addresses two major limitations: the dependence on animal-derived components and the generation of nutrient-rich waste. First, we evaluated the use of a microalgae-derived serum (MS) as a functional substitute for fetal bovine serum (FBS) in culture media. Results demonstrated that fish (MS4 and MS5; Figure 2A) and mammalian (MS3; Figure 2B) cell lines maintained comparable proliferation rates with up to 75% and 99.6% replacement of FBS by MS, respectively. These findings highlight the potential of microalgal-derived nutrients to effectively supply essential growth-supporting factors

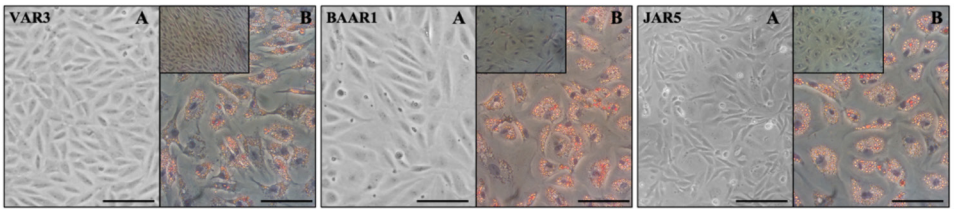


**Figure 1. Characterization of meagre cell lines derived from vertebra (VAR3), branchial arches (BAAR1) and jaw (JAR5).** (A) Phase-contrast images of proliferating cells in culture. (B) Adipogenic differentiation assessed by Oil Red O staining; lipid droplets appear in red. Insets show undifferentiated control cells. Scale bars: 100 µm.

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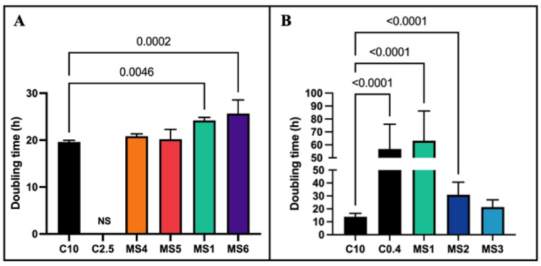


**Figure 2. Effect of microalgae serum (MS) on the proliferation of fish (A) and mammalian (B) cell lines.** Graphs show the population doubling times of cells cultured in medium supplemented with either FBS or MS. C10: medium with 10% FBS; C2.5 / C0.4: media with 2.5% or 0.4% FBS. MS1–MS6: media with different MS formulations. P-values (unpaired t-test) indicate significant differences between C10 and experimental conditions. NS: no cell survival.

**Table 1. Bioremediation of cell culture effluents using microalgae.** Ammonia, nitrate/nitrite and phosphates concentrations in the spent cell media before and after microalgal cultivation. Superscript letters denote statistically significant differences between pre- and post-bioremediation values (a = $p < 0.05$; b = $p < 0.001$).

| | Before bioremediation | After bioremediation | % of reduction |
|---|---|---|---|
| **Ammonia (mmol/L)** | 1.948 ± 0.012 | 0.356 ± 0.411[a] | 81.72% |
| **Nitrates/nitrites (mmol/L)** | 0.024 ± 0.002 | 0.007 ± 0.001[a] | 70.83% |
| **Phosphates (mmol/L)** | 0.902 ± 0.004 | 0.353 ± 0.125[b] | 60.86% |

Then, we explored the potential of spent cell culture medium as a substrate for microalgae cultivation. Microalgae cultured in spent medium exhibited significantly enhanced growth, achieving a final dry weight of 2.468 ± 0.413 mg/mL by day 7, compared to 0.915 ± 0.436 mg/mL in control medium. Moreover, microalgae grown in spent medium displayed a significantly higher nitrogen content (6.03 ± 2.73%) than those in control medium (1.82 ± 0.25%). Cultivation in spent medium also resulted in substantial reductions in residual nutrients - including ammonia, nitrites/nitrates, and phosphates -indicating efficient nutrient uptake and bioremediation (Table 1). These findings underscore the capacity of microalgae to valorize nutrient-rich effluents and contribute to the sustainable recycling of cell culture medium waste.

## Conclusion

Our results demonstrate the feasibility of combining marine animal cell culture with microalgal systems to foster a circular, zero-waste model for cellular aquaculture. Together, the establishment of robust meagre cell lines and the integration of microalgae-based bioprocessing lay the foundation for a scalable, ethical, and resource-efficient platform for the production of cultivated seafood.

## Funding

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# SUSTAINABLE BIOCHAR SUPPLEMENTATION IN AQUAFEEDS: IMPLICATIONS FOR WATER QUALITY AND METABOLIC RESPONSE IN SENEGALESE SOLE (*Solea senegalensis*)

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## Introduction

Biochar is a carbon-rich material synthetized by the pyrolysis of biomass under oxygen-poor atmosphere. Among its many potential applications, its inclusion as a feed additive in aquaculture has gained attention due to promising results. Its porous structure and strong adsorption capacity have been associated with improved nutrient uptake, which may enhance feed utilization and promote better overall fish health (Amjad et al., 2024). The retention capacity could help trap nitrogenous compounds, thereby reducing their concentration in the water (Khalid et al., 2024). In addition to its functional advantages, using biochar derived from wood waste supports circular economy strategies, encouraging more sustainable production practices and potentially lowering the reliance on conventional ingredients like fish meal and fish oil. Within this framework, the aim of the present study was to assess the metabolic impact of dietary biochar supplementation in Senegalese sole (*Solea senegalensis*), focusing on an inclusion level that maintains growth and health.

## Material and methods

The biochar was produced on an industrial scale through the carbonization of plum (*Prunus domestica*) residues at around 450 °C.

A total of 270 Senegalese sole (*Solea senegalensis*) (initial mean body weight ~65 g) were randomly distributed into 9 tanks (30 fish per tank, in triplicate), ensuring similar initial biomass. Fish were fed three times daily with a ration equivalent to 1% of the estimated tank biomass (adjusted weekly), using three experimental diets: i) control, ii) supplemented with 0.25 % biochar, and iii) supplemented with 0.50 % biochar. The sole were reared for 84 days at the facilities of the Central Marine Culture Research Service of the University of Cádiz.

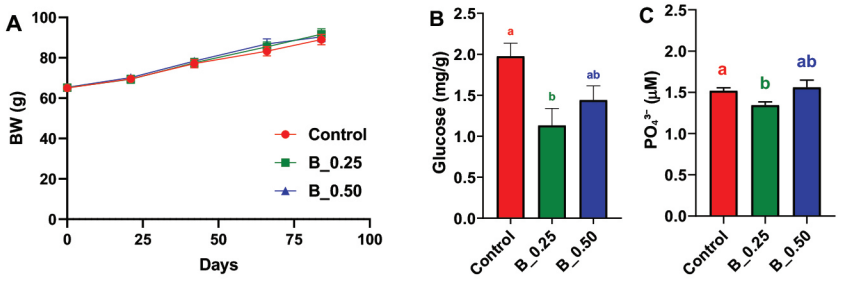


**Fig.1: A)** body weight (BW); **B)** muscle glucose and **C)** water $PO_4^{3-}$ levels at the end of the trial.

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During the initial sampling, all fish were chipped to allow individual growth monitoring, adding robustness to the dataset. Intermediate biometric samplings were conducted every 21 days to track growth evolution. Water samples were collected over 5 consecutive days at 2 time points: one prior to feeding (low nitrogen compounds levels) and another 9 h after the first intake (high nitrogen compounds levels). At the end of the feeding trial, biometric data were recorded, and plasma, liver, and muscle samples were collected. Somatic indexes were calculated, and cortisol and metabolite levels from each tissue were analysed, along with the determination of phosphate, silicate and nitrogenous compounds in water samples.

**Results and discussion**

The inclusion of biochar at 0.25–0.50% did not impair overall growth performance of Senegalense sole (Figure 1A). However, a significant improvement in SGR was observed during the third period at 0.25%, along with positive trends in final BW, and overall SGR and FCR. ILI and HSI also tended to decrease, particularly with 0.25%. The shortening of the intestine may result from improved absorptive efficiency, induced by biochar, while the reduction in liver size could reflect greater metabolic optimization.

Although plasma and liver metabolites showed no significant changes, some trends were observed. Hepatic glycogen and glucose levels appeared to decrease, possibly due to their use in *de novo lipogenesis* for TAG synthesis, which tended to increase in both liver and plasma. Alternatively, these reductions, along with the slight decrease in plasma protein levels, may reflect increased metabolic demands in muscle, where significant decreases in energy-related metabolites (e.g. glucose Figure 1B) were found, potentially supporting enhanced growth. This effect was most evident with the 0.25% diet, which recorded the highest final BW, yet not statistically significant. Cholesterol levels remained unchanged in plasma and liver but increased significantly in muscle with 0.50% biochar, possibly reflecting membrane remodelling or shifts in lipid composition. Plasma lactate slightly increased with 0.25% biochar, likely due to greater muscle activity. Cortisol levels also showed a modest increase, possibly related to physiological adaptations such as intestinal shortening. However, both parameters remained below stress-indicative thresholds, suggesting only mild physiological adjustments.

Finally, the significant reduction in $PO_4^{3-}$ (Figure 1C) and downward trends in $NH_4^+$ and $SiO_2$ levels in water of specimens fed the experimental diets points to potential environmental advantages of incorporating biochar.

Short-term inclusion of biochar appears to induce promising functional effects, particularly at 0.25% supplementation. Longer trials are required to explore the implications not only in microbiota and enzymatic activity, but also its interaction with other additives such as microalgae.

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# HARNESSING POSTBIOTICS FOR SUSTAINABLE HEALTH MANAGEMENT IN SHRIMP AQUACULTURE

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Shrimp aquaculture, a rapidly expanding sector of global seafood production, faces critical challenges related to disease outbreaks, antibiotic overuse, and environmental sustainability. Postbiotics—non-viable microbial cells, metabolites, and cell components conferring health benefits—have emerged as a promising alternative to traditional antimicrobials and probiotics. Unlike probiotics, postbiotics offer enhanced stability during feed processing and storage and pose no risk of horizontal gene transfer or uncontrolled microbial proliferation in the aquatic environment.

Two recent studies were conducted to evaluate the efficacy of dietary postbiotics in improving shrimp (*Litopenaeus vannamei*) resilience under Early Mortality Syndrome (EMS) and White Spot Syndrome Virus (WSSV) challenges, including exposure to salinity stress. In both trials, shrimp were fed a basal diet supplemented with postbiotics and assessed following pathogen exposure under controlled experimental conditions.

Postbiotic-fed groups showed significantly higher survival rates and improved outcomes under both EMS and WSSV challenge models. In the EMS trial, reduced mortality and better intestinal and hepatopancreatic condition were observed. Under WSSV and salinity challenge, shrimp exhibited enhanced resistance and lower stress-induced susceptibility. Histological analysis also indicated improved gut integrity, supporting overall robustness under environmental and infectious stressors.

These findings support the integration of postbiotics as a viable functional feed additive in shrimp aquaculture, contributing to disease prevention and production efficiency. As regulatory frameworks evolve and consumer demand for antibiotic-free aquaculture intensifies, postbiotics offer a sustainable solution aligned with industry trends. Future work should focus on optimizing postbiotic formulations and elucidating molecular mechanisms underlying their beneficial effects in shrimp and other aquaculture species.

# TOWARDS *Salmo carpio* AQUACULTURE: PRELIMINARY ASSESSMENT OF THE USE OF FUNCTIONAL INGREDIENTS IN DIET

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## Introduction

Carpione del Garda (*Salmo carpio*, hereafter carpio) represents a unique element of European ichthyofauna. This salmonid fish is strictly endemic to Lake Garda, the largest lake in Italy, meaning it naturally occurs nowhere else. Moreover, carpio is listed as endangered species by International Union for Conservation of Nature due to the rapid decline of wild population. Therefore, several conservation actions, including the exploration and development of aquaculture techniques, have been attempted so far. There are several aspects that must be properly addressed in carpio farming, including optimal diet formulation, welfare assessment, fillet quality, health management, definition of proper larval-stage and weaning protocols.

The project "Filiera ASTRO: Competitiveness and Sustainability of Mountain Aquaculture" (CUP J38H23001450007), funded by "Piano Nazionale Complementare – PNC" of the Ministry of Agriculture, Food Sovereignty and Forests (MASAF), aim to improve the local aquaculture industry through the reuse of fish farming by-products, and applying new technologies to reduce feed waste and environmental impacts. The project framework consists of four work packages (WP). The present activity has been conducted within the WP1 which is focus on developing farming protocol for carpio, improving animal survival through larval weaning techniques, studying factors for successful sexual maturation and reproduction to enhance fecundity and hatching rates. WP1 will also include a genetic characterization of the breeding stock to ensure genetic purity and establish a suitable management plan.

In this context, the use of functional ingredients might help in supporting growth and health status of fish in farming condition. The aim of this work is to assess the use of functional ingredients in carpio's diet evaluating the effect on growth performances and other common indicators in aquaculture. The same diet was tested simultaneously on rainbow trout (*Oncorhynchus mykiss*). In the present work, only data referring to carpio will be reported.

## Material and Methods

The trial was conducted at the fish experimental facility of Fondazione Edmund Mach (San Michele all'Adige, TN, Italy). The trial was conducted in 700 l flow-through tanks (24 water renewal per day). Water parameters were maintained at a temperature of 12.5 °C, pH 7.85, and oxygen saturation between 80% and 95%. The fish were exposed to natural photoperiod (46°11'37"68 N; 11°8'4"56 E).

A total of 180 carpio (mix sex; 1+ year old. The average initial body weight was (mean ± SD) 120.5 ± 21.7 g for carpio. Fish were randomly distributed into three tanks per group, with 30 fish per tank. Two dietary groups were compared: a control diet (Vita 3, Veronesi, Verona, Italy) and a treatment diet (Vita 3, Veronesi, Verona, Italy) supplemented with 5 g $kg^{-1}$ of functional ingredient (GillFish Professional, Greenvet, Italy). Diet was supplemented according to feed manufacturer specifications from 1.8 % to 1 % of fish biomass in each tank. Fish were fed four times per day, five days per week, until apparent satiation. Any feed leftover was collected and quantified.

Growth performance and feed efficiency were evaluated through the following parameters: mortality rate (MR), mean final body weight (FBW), Condition factor (K) specific growth rate (SGR), feed conversion ratio (FCR) and feed intake (FI).

Data was compared between groups within species using the most appropriate statistical test. The detail of statistical test used is reported in Table 1. The software used for statistical analysis was R.

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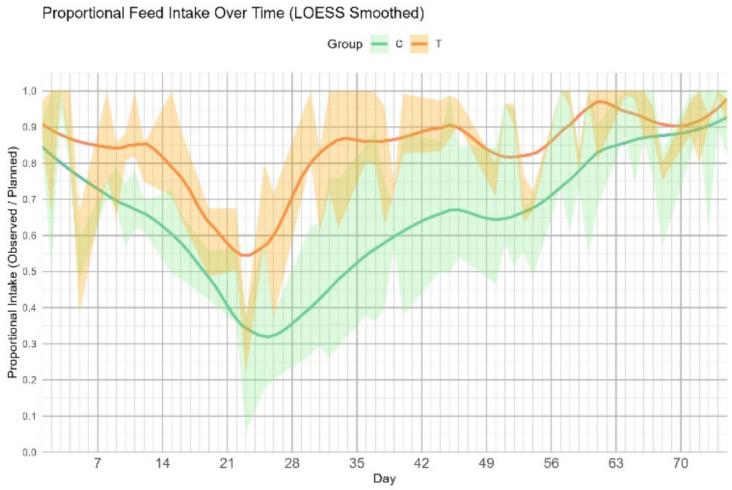


Figure 1. Comparison of relative daily feed intake (apparent/planned) in carpio fed either a control (C, green line) or treatment (T, red line) diet. Data are presented as smoothed mean (solid lines) with shaded areas indicating the minimum and maximum feed intake across replicate tanks.

**Results and Discussion**

The results showed that the use of functional ingredients in carpio diet improved final body weight (Two samples T-test, $P < 0.001$, $157.9 \pm 39.0$ and $178.1 \pm 34.9$ in control and treatment group, respectively), and K (Two samples T-test, $P < 0.001$, $1.19 \pm 0.11$ and $1.28 \pm 0.12$ in control and treatment group, respectively). The mortality rate observed in carpio fed treatment diet (5.6 %) was lower compared to those fed on control diet (13.3 %). One of the main bacterial diseases affecting carpio is the cutaneous flavobacteriosis. During the trial, both fish in the control and treatment group were affected, but the severity of the infection resulted lower in those fish fed on treatment diet. Regarding feed consumption no statistical differences have been observed between both group despite the highest FCR observed in control group ($2.57 \pm 0.85$) than in treatment group ($1.43 \pm 0.10$). As showed in Figure 1, the treatment group showed a significantly higher (Generalised linear mixed-effect model GLMM, adjusted for repeated measures of the same tank; $p = 0{,}004$) relative feed intake (apparent/planned) compared to control group over time. Towards the end of the experiment, feed intake in the control group increased, eventually overlapping with that observed in the treatment group. This suggests that the use of functional ingredient in carpio could be useful during specific stages of farming (e.g., manipulation, sorting, etc.) while in case of long-term exposure, benefits could not be so evident.

**Conclusion and future perspectives**

The present study offers preliminary insight on the use of functional ingredients in carpio. The growth performances and survival rate resulted increased when functional ingredients are used. Despite beneficial in terms of growth rate and feed intake, the addition of functional ingredients represents and additional cost to fish farmers. Therefore, the trade-off between growth, survival, and costs should be properly evaluated in case of long-term exposure to functional ingredients. Considering the observed positive effects, additional data (e.g., gene expression and histological analysis) are needed to further support the present findings.

# GROWTH PERFORMANCE AND ROBUSTNESS OF ATLANTIC SALMON (*Salmon salar*) FED DIETS CONTAINING VARYING COMBINATIONS AND INCLUSION LEVELS OF TWO DIFFERENT MICROBIAL INGREDIENTS (*Paecilomyces variotii* and *Schizochytrium sp.*)

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**Introduction**

The aquaculture industry is faced with several challenges, including access to high quality feed ingredients, and exposure to multi-stressor conditions such as changing environmental conditions and diseases leading to mortalities and economic losses. Therefore, there is an urgent need for novel, sustainable feed ingredients that can also improve the health and robustness of the fish. Microbial ingredients are viable alternatives to address the shortage of both protein and long-chain omega-3 fatty acids. Furthermore, many microbial ingredients contain bioactive components such as β-glucans, mannans and nucleic acids which can improve robustness and health of fish.

*Paecilomyces variotii* (PEKILO®) is a filamentous fungus that can be fermented on forestry side-streams and has promising nutritional properties for use in aquafeeds. *P. variotii* has a high protein content of 60-70% (dry matter basis) with a favorable amino acid composition and has shown to be rich in β-glucans and nucleotides (Hooft et al., 2024), which promote immune modulation and growth. Mensah et al. (2024) observed that spleen leukocytes exposed to *P. variotii* and *Moritella viscosa* showed up-regulation of proximal pro-inflammatory cytokines *Il1β* and *tfnα*, suggesting that *P. variotii* induces pro-inflammatory responses. Additionally, spleen leukocytes co-stimulated with *P. variotii* and *M. viscosa* showed immune homeostasis, with higher gene expression of *il6* and *tgfβ* after 48 hours. These observations indicate that *P.variotii* can potentially boost immune responses and produce a more robust fish.

Another microbial ingredient is *Schizochytrium sp.,* which is a microalgae specie that is high in the essential omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Santigosa et al., 2020) and has been shown to improve mucosal barrier of skin, gills and intestine of Atlantic salmon and can replace up to 50% of fish oil (Islam et al., 2024). EPA and DHA have essential roles in many biological processes and properties like the cell membrane and cellular synthesis, and both the level and ratio of EPA and DHA have effects on the health of Atlantic salmon (Berge et al., 2019; Lutfi et al., 2023; Santigosa et al., 2023).

**Methods and materials**

In this experiment, vaccinated pre-smolt Atlantic salmon has been fed five different diets containing varying inclusions of *P. variotii* and *Schizochytrium sp.* in freshwater tanks for four weeks. Currently, the fish is being transferred to saltwater tanks where they will be exposed to the winter ulcer causing pathogen *M. viscosa*. The experiment will be concluded early June this year, and a wide range of samples will be collected.

We will present the main effects of feeding diets containing varying inclusions of *P.variotii* and *Schizochytrium sp.* for four weeks in fresh water and the effects on growth performance, robustness and health of salmon after being transferred to salt water then exposed to pathogens.

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*Table 1. Diets and inclusion level based on crude protein and crude fat*

| Diet | Inclusion |
|---|---|
| Diet 1 | 0% *P.variotii* + 0% *Schizochytrium sp.* |
| Diet 2 | 10% *P.variotii* + 5% *Schizochytrium sp.* |
| Diet 3 | 20% *P.variotii* + 10% *Schizochytrium sp.* |
| Diet 4 | 0% *P.variotii* + 10% *Schizochytrium sp.* |
| Diet 5 | 20% *P.variotii* + 0% *Schizochytrium sp.* |

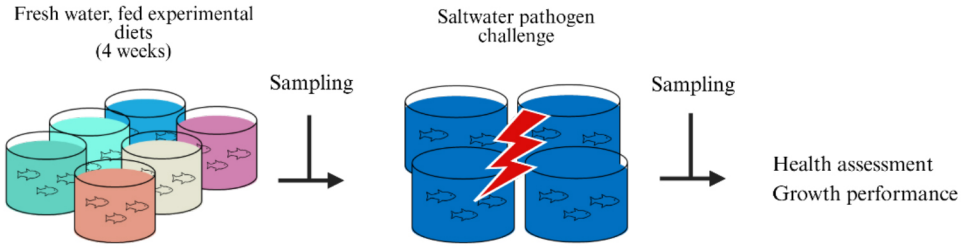


Figure 1. Experiment set-up. Fish fed experimental diets with varying inclusion levels of *P.variotii* and *Schizochytrium sp.* for four weeks in freshwater tanks, transferred to saltwater tanks and exposed to *M.viscosa*.

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# INVESTIGATING THE HUMANE KILLING OF *Homarus americanus* THROUGH SCIENCE-BASED BIOMARKERS

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**Introduction**

The European Commission (EC) Regulation 1099/2009 states that animas shall be spared any avoidable pain, distress, or suffering during killing and related operations. This is likely since slaughter is one of the most critical moments in the life of animals caught or reared for food, during which their welfare can be severely compromised. In crustaceans, sufficient evidence of pain perception is still under debate. Nonetheless, several studies have shown that crustaceans possess a well-developed nervous system, nociceptors, and exhibit responses to noxious stimuli that go beyond simple reflexes (Crump et al., 2022). According to EFSA (2004), signs of pain and distress in animals should, whenever possible, be confirmed using animal-based indicators (ABIs), such as physiological biomarkers. Based on these indicators, strategies such as an adequate electrical stunning could be developed to mitigate pain in the American lobster (*Homarus americanus*) prior to slaughter. The aim of this study was to evaluate changes in a panel of substances, some measured for the first time in this species, following an electrical stunning process. This could contribute to the identification of potential biomarkers that may improve both animal welfare and meat quality.

**Materials and methods**

Male lobsters were acclimated for four weeks in six fibre tanks (2,000 × 1,000 mm) with individual compartments in a cold chamber with running seawater at 12°C and 12:12 photoperiod of dark and blue light. They were fed mussels throughout the rearing period. Lobsters were divided into two groups: a control group (C, $n = 19$) and a stunning group (TL, $n = 18$). To induce immediate unconsciousness prior to slaughter, lobsters in the TL group were subjected to electrical stunning using the commercial Crustastun™ device (Mitchell & Cooper Ltd, UK). The standard long cycle (10 seconds at 8 A, 220 V) was applied, following manufacturer's operational procedures. Lobsters in the control group (C) were placed in the Crustastun™ with no electrical current. After stunning, lobsters were kept on ice for 30 min prior to dissection, to sedate the animals. Haemolymph was extracted and immediately freezed in $N_2$ (l) and later preserved at -80ºC using standardized protocols (Lorenzo et al., 2024). Biochemical parameters measured in haemolymph included glucose, lactate, L-lactate dehydrogenase (LDH), urea, triglycerides, cholesterol, total proteins, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and D-dimer. Analyses were conducted at the Interdisciplinary Clinical Analysis Laboratory of the University of Murcia (Interlab-UMU) using an Olympus AU600 automated biochemical analyser (Olympus Diagnostica GmbH AU600) with commercial kits from Beckman Coulter Inc. and Diazyme Laboratories (CA, USA). Differences in biomarker levels between groups were assessed using the non-parametric Mann–Whitney U test for unpaired samples, performed with GraphPad Prism 9 (La Jolla, CA, USA). A $P < 0.05$ was considered statistically significant.

**Results and discussion**

The biomarkers that showed the greatest changes between groups are shown in Figure 1; the rest of the biomarkers did not show significant differences.

There is little data on this species in relation to the assessment of pain and stress after electrical stunning, increase in metabolites such as glucose and lactate have previously been described in stunned lobsters also showing that lactate was a better indicator than glucose (Lorenzo et al. 2024). Increases in triglycerides have also been reported in other crustacean species (Coneely and Coates, 2023) in response to stress. However, there are no data on D-dimer, and this is the first time it has been determined in this specie. D-dimer is a peptide found is derived from the degradation of blood clots and significant increases has been found in species as pig after stress (López-Martínez et al., 2020).

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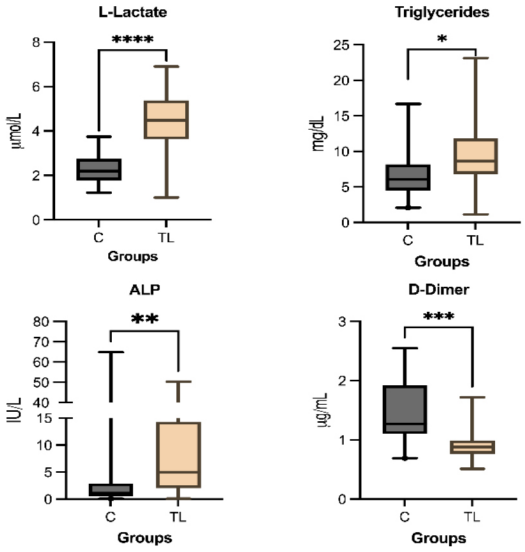


Figure 1. L-Lactate, triglycerides, alkaline phosphates (ALP) and D-dimer values in haemolymph obtained from control (C; n = 19 lobsters) and stunning group (TL; n = 18 lobsters). Graphs show medians (line within box), 25th and 75th. percentiles (boxes), min and max values (whiskers) and individual values (points). Asterisks indicate statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and **** $P < 0.0001$).

**Conclusion**

Significant differences were observed in lactate, triglycerides, ALP, and D-dimer, biomarkers linked to different aspects of physiological response. While further research is necessary, these parameters show potential as indicators for evaluating the effects of electrical stunning on animal welfare and possibly the future quality of lobster meat.

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# PERCEPTION OF CHALLENGES AND NEEDS REGARDING ANIMAL WELFARE IN LATIN AMERICAN AQUACULTURE

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**Introduction**

Animal protein production faces challenges such as intensifying farming practices, boosting feedstuff yields, and reducing environmental impacts to meet UN sustainability goals. Ethical concerns about animal welfare, recognizing animals as sentient beings, have pressured producers, legislators and consumers to adopt humane farming practices. This study examines Latin American aquaculture stakeholders' perceptions in order to address issues ensuring the four domains of animal welfare: nutrition, environment, behavioural interactions and health freedom from hunger, discomfort, pain, and the ability to express normal behaviour.

**Methods**

Aquaculture stakeholders from Latin American Countries (scientist, national administrations and private sector), participated in a 3-days Aquatic Animal Welfare (AAW) workshop within the framework of the CEREBAL project, with a total of 150 respondents. Focus group interviews encouraged participants to respond to the question: *what problems can be found in aquatic-animal welfare?* across four dimensions (good nutrition GN, appropriated housing AH, appropriate behaviour AB and good health GH), aquaculture processes (developmental stages, culture methods transport and slaughter) and eight species (tagging responses to research, legislation, or operational practices). A Latent Dirichlet Allocation (topic modelling analysis), was performed for Spanish words, revealing main topics and the most frequent terms used for describing each dimension and category problems.

**Results**

Participant's answers in the developmental stages category highlighted three latent topics: 1) animal-based welfare indicators, 2) feeding strategies, and 3) sanitary conditions. Frequent terms across dimensions included "food", "stress" and "mortality", while "cannibalism" was related to early life stages. Reproductive stage emphasized "spawning", "stress" and "females", while grow-out stage featured "feeding" and "sanitation". When comparing different culture systems, the main topics of GN were: 1) the availability of appropriate quality food, 2) food distribution, and 3) feeding strategies, using "distribution", "food" and "bottom" as more frequent words. The main topics identified in AH dimension were: 1) water quality and density, 2) environmental variables and 3) predator entry to the facility. The words "density" and "environmental" and "predator" were prevalent in all categories. The GH dimension featured: 1) availability of veterinary healthcare, 2) water control, 3) disease control and appropriate diagnose, as main topics. The most frequent words used within these topics included: "difficulty", "diagnose", "control", "pathogen" and "presence". Recirculating Aquaculture Systems included "correct", "veterinary" and "treatment", whereas the floating cages systems related more to "presence", "environmental" and "disease". The AB dimension answers, according to culture systems, showed: 1) behaviour assessment, 2) appropriate facilities and 3) natural behaviour in production; as the most relevant topics. Words choice indicated general and ambiguous responses, suggesting limited participant knowledge. Main topics in the slaughter methods category featured: 1) capacity building, 2) avoid stress, suffering and pain during procedures, 3) procedures in tanks may cause suffering and stress. "Training" (as in capacity building) was the

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Figure 1: Most frequent words showcase “behaviour”, “diagnose” and “disease” for Research needs; “pharmacological”, “legislation” and “traceability” for Rules and regulations whereas “management”, “handling” and “food” for Operational practices.




leading word across responses and dimensions. The GN included words such as “fastening” and “stress”. The words “tank” and “overcrowding” appeared recurrently in AH. AB included “method” and “protocol”, whereas GH included “stress”, “suffering” and “pain”. Slaughter methods featured words as: “type”, “adequate” “concentration” (for anaesthesia); “need”, “effectiveness” and “training” (electric shock); “suffering”, “procedure” and “protocol” (for ice). The responses to species-specific problems showed the need to develop welfare indicators based on the animal. It showcased topics such as 1) nutritional needs, 2) species- specific behaviour, 3) feeding strategies and 4) disease diagnose. The needs for research include: nutrition and feeding, behaviour, stress, water management and environment. Rules and regulations should address key aspects on: food, production facilities, animal health, capacity building and environmental impact. Operational practices showed: management and animal handling, standardized protocols, personnel training and comprehensive guidance documents. The most frequent words used are displayed in Fig.1.

**Discussion and Conclusion**

In general, Latin- American stakeholders show concern of topics mainly related to increase sustainability in their process, and a deep need to develop theoretical and technical abilities to address issues related to welfare concerns such as stress, pain and suffering. As each species has unique needs and challenges, it is necessary to establish interventions that result in effectively distinguish the best conditions for improving welfare (1). We hope that these data can raise awareness of Aquatic Animal Welfare issues and promote innovation and development to address local issues and be translated into laws and guidelines that guarantee the welfare of farmed aquatic organisms.

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# IS CHILLED WATER ANAESTHETIZING WHITE-LEG SHRIMPS, *Penaeus vannamei*?

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## Introduction

The white leg shrimp, *Penaeus vannamei* is the worldwide leader of aquaculture production for crustaceans, with over 7 million tonnes farmed in 2022 alone (FAO, 2024). Production at these volumes can lead to poor welfare standards, especially if done in remote or inaccessible places. Welfare standards include the best practices for manipulation and before euthanasia, especially in farms with large volumes of production.

However, for shrimps, the effect/response to anaesthetics is vaguely known (Rotllant et al., 2023). A newly developed definition should tackle EFSA's triangulation principle, correlating the organisms' responses at different levels of organisation. Farm standard practices involve using slurry ice as an anaesthetic before dispatch, although its effect remains unknown. In this work, we conducted a three-part experiment to characterize behavioural, metabolic, and neurophysiological responses to 'cold' seawater (~10°C) as an anaesthetic to determine if this practice reduces this shrimp species' neural and physiological activities.

## Materials and methods

Juveniles of *P. vannamei* of 21.1±1.3 mm carapace length (CL) and 6.3±0.5 g wet weight (ww) were delivered to the aquaria facilities of the Autonomous University of México (UNAM), Sisal, and left for two weeks at 24°C and 34UPS. Animals were fed commercial pellet food daily and maintained under a 12L:12D period. The working temperature was 10°C, defined as the thermal condition in which animals were rendered unresponsive in less than 10 min and recovered their normal position in under 20 min.

The first set of experiments on the effects of temperature focused on the behavioural responses of shrimp, particularly aiming to find the time when animals remain at the bottom of the tank lying on their side, and after were unresponsive when stimulated by touching the legs. This last marked the desired response and after which water was changed to let the animals recover (at 24°C). Animals were sampled at the end of the anaesthesia stage (n=7), or the end of the recovery (n=7) to measure metabolic and immunologic stress markers in haemolymph, muscle and midgut gland.

The second set of experiments focused on the effects of temperature on the metabolic rate (MR) of *P. vannamei*. The oxygen consumption ($VO_2$) was measured using a continuous flow respirometer of a closed chamber connected to a recirculating seawater system. Animals were placed in the respirometry chamber 12h before the experimental measurements, with a shrimp-less chamber used as a baseline. Once 120 min of baseline was recorded, the cooled seawater was added to the system, allowing the temperature inside the respiratory chambers to drop to ~10°C after 17 min. After 10 min of exposure, the system was refilled with seawater at 24°C. Metabolic rate was measured after, during, and before the seawater temperature change.

Lastly, neurophysiological activity and heart rate were recorded. Electrodes were implanted flanking the supraesophageal ganglion to measure electroencephalogram (EEG), visual evoked responses (VERs), and flanking the pericardial zone to measure heart rate (HR). Once a baseline was measured, water was changed to add the anaesthetic (cold water, ~10.5°C) and measures continued for 5 min, before water was changed again, to measure the parameters of recovery.

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Analysis of variance (ANOVA) was used to evaluate if there were differences in each experimental process; analyses were carried out using R version 4.4.2.

**Results**

Exposing animals to cold water induced an almost immediate equilibrium loss, after several body spasms, and, unresponsiveness after 1.3±0.3 min. The recovery happened after 11.0±5.9 min in renewed seawater. The haemolymph lactate levels remained unaltered after anaesthesia (initial, 0.060±0.053 mg/mL vs unresponsive, 0.141±0.062 mg/mL). Interestingly, the lactate levels were significantly increased after recovery (0.422±0.025 mg/mL, $p < 0.0008$) when compared to the original levels. The opposite is seen for cholesterol in the midgut gland, where it decreased after the recovery (initial, 0.815±0.023 mg/mL vs. recovery, 0.668±0.016 mg/mL, $p<0.05$). The MR of animals exposed to cold seawater, was 20% lower than the MR before the cold exposure. After recovery, the MR remained 3% higher than before exposure to cold water.

During the first minute of exposure to cold sweater (~10.5°C) VERs were lost, and remained undetected for the 5 min. Once the water was changed, animals recovered VERs within the first minute. Similarly, the HR had a sharp drop (ca.50%) in the first minute of exposure, dropping to 35% of the basal response (168±17 bpm vs 59±11 bpm, baseline vs cold seawater). Interestingly, the recovery of the HR took about 2 min, but it remained 16% lower than during the baseline (168±17 bpm vs 141±31 bpm).

**Discussion and conclusions**

During experimental trials, the loss of equilibrium was expected (called 'stage II') and the following stage (III), both characteristics previously described as inactivity of the cephalothoracic appendages and lack of response when stimulated (De Souza Valente C., 2022). Exposure to 'cold' (~10.5°C) seawater had animals losing their balance almost instantly, although they performed some vigorous escape response before it. During the respirometry analysis some animals lost their balance, displaying vigorous movements since the drop in temperature happened progressively (17 min). In such a condition, the physiological stress was revealed, showing mobilization of reserves and increased lactate.
On a neurophysiological level, the loss of the VERs could indicate the desired anaesthetic stage. The sharp drop in the HR reinforces the idea that cold water could be and effective anaesthetic. It is necessary to point out that the temperature chosen is around this species' minimum critical temperature ($CT_{min}$). This could explain the increase in lactate (and metabolic rate) during recovery. Acute exposure to near CTmin temperature could instantly achieve the desired anaesthesia stage, since some sensorial pathways are shut down. Yet, other organizational levels could be irreversibly damaged and only be seen after a late recovery of *P. vannamei*. The effect of cold water studied on the different levels of organization evaluated show that shrimps were quickly anesthetized, however their recovery was as well quick. Hence, we suggest that the use of slurry ice in farms is followed by a quick slaughter process.

**Acknowledgments**

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# IS THE OREGANO OIL AN ANAESTHETIC FOR THE WHITE-LEG SHRIMP, *Penaeus vannamei*?

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## Introduction

The white leg shrimp, *Penaeus vannamei* is the worldwide leader of aquaculture production for crustaceans, with over 7 million tonnes farmed in 2022 alone (FAO, 2024). Production at these volumes can lead to poor welfare standards, especially if done in remote or inaccessible places. Welfare standards include the best practices for manipulation and before euthanasia, for which the use of sedatives or anaesthetics is recommended. An ideal anaesthetic should be affordable and readily available in most places, with known working concentrations (Rotllant et al., 2023). Yet, for this group of animals, the effect/response to anaesthetic is vaguely known (De Souza Valente, 2022). A newly developed definition should tackle EFSA's the triangulation principle, correlating the organisms' responses in different levels of organisation. In this work, we conducted a three-part experiment in order to characterize behavioural, metabolic and neurophysiologic responses to oregano oil as an anaesthetic.

## Materials and methods

Juveniles of *P. vannamei* of 21.1±1.3 mm carapace length (CL) and 6.3±0.5 g wet weight (ww) were delivered to the aquaria facilities of the Autonomous University of México (UNAM) in Sisal, and left for two weeks at 24°C and 34UPS. Animals were fed commercial pellet food daily and maintained under a 12L:12D period. The working concentration of oregano oil was established once animals were rendered unresponsive in less than 10 min, and were able to recover their normal position under 20 min. The first set of experiments focused on evaluating the effects of oregano oil (150 $\mu$L/L) on shrimp's behavioural responses. It aimed to find out when animals remained on the bottom of the tank, lying on their sides, and were unresponsive when stimulated by touching the legs. This last marked the desired response, and after which water was changed to let the animals recover. Animals were also sampled at the end of the anaesthesia (n=8) and recovery (n=9) to measure metabolic and immunological stress markers in haemolymph, muscle and digestive gland. The second set of experiments focused on the effects of oregano oil (150 $\mu$L/L) on the metabolic rate (RMR) of *P. vannamei*. The oxygen consumption ($VO_2$) was measured using a continuous flow respirometer of a closed chamber connected to a recirculating seawater system. Animals were placed in the respirometry chamber 12h before the experimental measurements, with a shrimp-less chamber used as a baseline. Once 120 min of baseline was recorded, the oregano oil was introduced into the chambers via de flow-through seawater system, allowing for a complete water change after 17 min. After 10 min of exposure, the system was refilled with aerated seawater, with constant change, and measures continued for 4 h. Metabolic rate was measured after, during, and before the seawater temperature change. Lastly, we recorded neurophysiological and heart rate parameters. Electrodes were implanted flanking the supraesophageal ganglion to measure electroencephalogram (EEG), visual evoked responses (VERs), and flanking the pericardial zone to measure heart rate (HR). Once a baseline was measured, water was changed to add oregano oil (150 $\mu$L/L) and measures continued for 6 min, before water was changed again, to measure during recovery phase.

Analysis of variance (ANOVA) was used to evaluate if there were differences in each experimental process; analyses were carried out using R version 4.4.2.

## Results

The oregano oil induced an equilibrium loss after 6.1±0.7 min, a complete unresponsiveness after 10.0±1.1 min, and recovered after 15.3±5.7 min. Before losing balance, animals experience multiple spasms, after which they lost equilibrium. After the

animals were unresponsive the lactate levels were increased by 10-fold (0.8±0.2 mg/mL), and after the recovery period they were still increased by 20-fold (1.7±0.3 mg/mL), when compared with basal levels (0.1±0.1 mg/mL). The RMR was 10% higher than observed in shrimp exposed to control seawater or ethanol (oregano's solvent). Yet, the recovery of the metabolic rate at the end of the experiment showed no difference. The exposure with oregano oil did not affect the VERs, since the beta waves amplitude during light stimulation was always distinguishable from dark or control periods (>10 ratio of magnitude), during and after the anaesthesia. For the HR, a slight decrease was observed from control levels during the exposure to oregano oil (a 17% drop). The HR returned to control levels after water change (~180 BPM).

**Discussion and conclusions**

As a starting point when doing trials with anaesthetics, the loss of equilibrium was expected, and noted as stage II, yet no other considerations (as what it took to be achieved) were taken into account. The following stage (III) of the anaesthetic involved the total inactivity of cephalothoracic appendices and lack of response when they were stimulated, while pleopod movement continued; cessation of pleopod movements resulted in no recovery (shrimp died). The exposure with oregano oil had animals lose their balance, but not before they performed an escape attempt from the aquaria. Similarly, during the respirometry analysis, after some time of avoidant response (or escape), animals lost their balance. The escape response could explain the higher metabolic rate found after the oregano oil was applied, and the loss of equilibrium could be a result of fatigue. This fatigue hypothesis could be reinforced by the unaffected VERs, since the loss of balance or inactivity does not involve any frontal ganglion dysfunction or failure on the primary sensory pathway. Interestingly, the mild drop in the HR is not consistent with a rise on the metabolic rate, but it is worth mentioning that the movement of these shrimps was restricted for electrodes placement and that could impact the oregano oil uptake. Overall, our results considering behavioural, physiological and neurological approach might indicate that oregano oil has not an anaesthetic effect on juvenile shrimps, although the animals have lost equilibrium after exposure. Therefore, without further results to ensure replicability and robustness, we will not suggest the use of oregano oil for shrimp aquaculture.

**Acknowledgments**

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# EVALUATION OF INCREASING LEVELS OF FERMENTED SOYBEAN MEAL AS A SUBSTITUTE FOR FISHMEAL IN THE DIETS OF JUVENILE EUROPEAN SEABASS (*Dicentrarchus labrax*) REARED UNDER FIELD CONDITIONS

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## Introduction

Due to the high cost of fishmeal and concerns about its limited availability and sustainability as the global aquaculture sector develops, research into suitable substitutes has been a longstanding priority. One of the most commonly used plant-based alternatives is soybean meal, which is generally regarded as the most nutritious protein feed for aquatic animals. Soybean meal has a relatively high protein content and a complementary blend of amino acids, making it suitable for inclusion in commercial fish feeds at levels of approximately 10-12%. The nutritional value of soybean meal (SBM) is often limited for cultured species due to deficiencies in methionine and the presence of antinutritional factors (ANFs).Various methods have been explored to reduce the adverse effects caused by these ANFs and specific oligosaccharides, with fermentation being a prominent approach of interest (Kumar et al., 2020). This study aimed to evaluate the potential of fermented soybean meal (FSBM) as an alternative protein source to fishmeal for the diets of European seabass (*Dicentrarchus labrax*) and to examine its effects on feed efficiency, growth performance, and immune response in a field trial under intensive aquaculture conditions.

## Materials and Methods

A 12-week trial was conducted to examine the effects of varying levels of fermented soybean meal (FSBM) (PERMEX VET Kft., Hungary) as a replacement for fishmeal (FM). Six diets were formulated to be isonitrogenous (46% crude protein) and isolipidic (16% crude lipid). These included a control diet containing 30% FM and three experimental diets that replaced FM with 30%, 50%, and 70% FSBM. All diets were produced as 3.5 mm pellets through a cooking extrusion process by SPAROS Lda (Olhão, Portugal). At the end of the feeding trial, the fish were collectively anesthetized, and 16 fish per cage were randomly sampled. Ten of these fish were used to assess proximate composition and fillet quality. From three additional fish, tissues from the anterior and mid intestines, along with the liver, were collected and immediately fixed in 10% buffered formalin for histological analysis. Finally, blood samples were collected from the caudal vein of three other fish per cage, and the serum was used for immunological assessments. All animal handling and sampling procedures adhered to the EU directive 2010/63.

## Results and Discussion

Overall, there were no statistically significant differences in growth performance and feed utilization indices ($P > 0.05$) among the experimental feeds. However, it is notable that the fish fed the 50% fishmeal (FSBM) diet exhibited the highest numerical growth parameters, including fish weight gain, specific growth rate (SGR), daily growth index (DGI), protein efficiency ratio (PER), and daily feed intake. In terms of feed conversion ratio (FCR), the lowest values were observed in cages fed diets containing 30% and 50% FSBM ($1.32 \pm 0.06$ and $1.33 \pm 0.03$, respectively). Similar to a previous lab trial (Roussos et al., 2023), the diet with 70% fishmeal substitution performed worse than the control, indicating limitations associated with higher inclusion rates of FSBM in the diets of European seabass. Furthermore, no significant differences were observed in the fish somatometric indices. However, the fish fed a diet containing 30% fermented soybean meal (FSBM) exhibited the lowest perivisceral fat index ($2.87 \pm 0.64$) compared to the control group ($3.42 \pm 0.59$). The overall body composition and fillet composition of the fish were not significantly affected by the substitution of FM with FSBM at any level of its inclusion. Nevertheless, a decrease in crude protein level ($18.24 \pm 1.57$, $18.93 \pm 1.32$, and $18.29 \pm 1.02$) was noted in the fillets of fish fed diets containing 30%, 50%, and

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70% FSBM, respectively, compared to the control group (19.42 ± 1.63). Additionally, the inclusion of FSBM did not impact the retention of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the fish muscle, as no significant differences were found. Moreover, no significant differences were found in serum total proteins, myeloperoxidase, and anti-proteases (% trypsin inhibition) among the different dietary treatments. However, protease activity was significantly lower in the diet containing 70% FSBM compared to both the control and the diet with 50% inclusion of FSBM. Also, a non-significant decreasing trend was observed in the serum lysozyme levels of fish that were fed diets containing increasing levels of FSBM. Lysozyme activity has been linked to bile acids (BA) concentrations, which play a role in regulating immune functions (Zhao et al., 2025). Additionally, BA have been associated with the presence of dietary taurine in feed. However, the substitution of fishmeal (FM) with plant proteins can lead to a decrease in taurine levels. It will be very interesting to further investigate the supplementation of fishmeal substitute-based feeds with taurine. Previous studies conducted by Kotzamanis et al. (2020a, 2020b) demonstrated that European seabass showed improved growth and fillet quality when fed a diet that included a mixture of soybean products and other plant meals, supplemented with 1% or 2% taurine as a replacement for fishmeal. These findings suggest that taurine is an essential nutrient that is often lacking in diets with high levels of soybean meal and other plant meal inclusions.

The results clearly demonstrated the high nutritional value of Fermented Soybean Meal (FSBM) for European seabass. In line with our previous trial, FSBM enabled the successful replacement of up to 50% of fishmeal without negatively affecting growth performance and feed utilization. However, it is important to emphasize that further research is needed to fully understand the effects of high inclusion rates of FSBM on the immune status of cultured fish.

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# MODULATION OF HEPATIC AND ANTERIOR INTESTINE GENE EXPRESSION IN EUROPEAN SEABASS (*Dicentrarchus labrax*) FED DIETS CONTAINING BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL AS AN ALTERNATIVE TO FISHMEAL

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**Introduction**

The use of insect meals in feed for farmed fish was recently approved by the European Commission (EU 2017/893). Over the past years, insect meals have gained significant interest, performing in a similar way to fishmeal-based diets (Piccolo et al., 2017; Ido et al., 2019). Insect larvae meals offer significant environmental and economic advantages. Insects reproduce and cultivate rapidly, requiring minimal water and land resources compared to other feed ingredients. They also facilitate waste biodegradation by transforming low-quality organic material into high-value nutrients, thereby contributing to enhanced soil fertility. Additionally, insects contain substantial amounts of high-quality protein and a balanced amino acid profile, which is superior to plant alternatives. Nevertheless, the potential of an ingredient should not be solely assessed based on its effects on growth performance. As with any novel feed ingredient, certain limitations merit further investigation. Previous research has indicated that the dietary inclusion of insect meals may impact intermediary metabolism across different species. Understanding the potential effects of dietary inclusion of insect meals on fish metabolism is also mandatory to improve fish nutrition as it may compromise fish growth performance and nutrient utilization, affecting fish health in the long term.

**Materials and Methods**

Black soldier fly (*Hermetia illucens*, BSF) larvae reared on plant-based residual substrates were experimentally produced and incorporated into aquafeed formulations, replacing fishmeal (30% incorporation in the control group - CTRL) at inclusion levels of up to 60%. A three-month feeding trial was carried out at the experimental facilities of the Hellenic Centre for Marine Research (HCMR), using European seabass (*Dicentrarchus labrax*, initial mean weight ~30 g). Fish were fed six experimental diets in which fishmeal was progressively replaced by black soldier fly larvae meal at 0 (CTRL), 20, 30, 40, 50, and 60% substitution levels. At the end of the trial, 108 tissue samples (liver and anterior intestine) were collected for molecular analyses. Gene expression profiling was performed using customized qPCR arrays targeting seabass markers that had been previously optimized at the IATS-ANA infrastructure. Several reference genes, including actb and ef1, were employed to normalize mRNA expression levels. A total of 58 target genes were examined, with 29 genes analyzed from each tissue type. More precisely, in the INT the expression profiling of genes related to antibacterial peptides (e.g., defb, leap2), mucus proteins (e.g., muc2), immunoglobulins and transcription factors (e.g., igms, nfkb2, il1b), T-cell markers (e.g., cd247, cd8b, ccr3), phagocyte activity (e.g., csf1r, mif, mmd), were evaluated. In the LIV, several lipid metabolism-related genes (e.g. elovl1, lpl, atgl) were investigated. The impact on fish growth was also assessed through the expression of hepatic genes related to growth hormone and insulin-like growth factor system regulation (e.g., ghr-i, igf-ii, igfbp1a).

The data normality and homogeneity of variances were checked through the Shapiro-Wilk and Levene's tests, respectively. In a normality and equal variance scenario, One-way ANOVA followed by a Tukey post hoc test was used to assess statistical differences ($p < 0.05$). Whenever data normality or homogeneity was not achieved, data transformation (i.e., logarithmic) or nonparametric statistical analysis (i.e., Kruskal-Wallis for gene expression, Spearman for correlation; $p < 0.05$) were employed. All statistical analyses were performed using SigmaPlot (version 15). To further explore tissue-specific gene expression patterns, partial least-squares discriminant analysis (PLS-DA) was performed using EZinfo v3.0 (Umetrics, Sweden), which enabled the visualization of multivariate clustering and discrimination among dietary groups.

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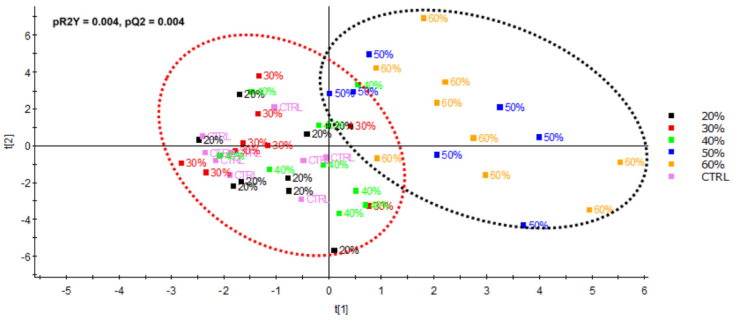


**Fig. 1** Multi-tissue PLS-DA

## Results and Discussion

No statistically significant differences in the expression of individual genes were initially detected among dietary groups in either tissue ($p > 0.05$). To further investigate potential dose-dependent effects across tissues, a partial least-squares discriminant analysis (PLS-DA) was performed by grouping the samples into CTRL–20–30–40 and 50–60. The PLS-DA model effectively discriminated between the two groups and identified 15 genes with a variable importance in projection (VIP) score > 1 as the primary contributors to class separation. In the anterior intestine, several immune-related genes were notably affected. Leap2 (Liver-expressed antimicrobial peptide 2), IL-8 (Interleukin 8), IL-22 (Interleukin 22), CSF1R (Macrophage colony-stimulating factor 1 receptor), and CD8b (T-cell surface glycoprotein CD8 beta) were upregulated in the higher inclusion group, suggesting enhanced immune activation and a shift towards a pro-inflammatory state. Conversely, the downregulation of CD4 (a T-cell surface glycoprotein) and IL-10 (Interleukin-10) indicated a reduction in regulatory and anti-inflammatory signaling.

These transcriptional changes underscore a potential compromise between immune activation and immune regulation in response to increased dietary incorporation of BSF larvae meal. Such responses could influence nutrient absorption, intestinal homeostasis, and overall health, thereby suggesting that inclusion levels approaching 60% may exceed the physiological tolerance of European seabass. Further investigation is necessary to identify strategies that may mitigate these effects, including biotechnological processing of insect meals to enhance digestibility or the addition of functional additives with immunomodulatory properties. These approaches could facilitate the optimization of insect-derived proteins, consequently reducing the aquafeed industry's dependence on fishmeal and advancing sustainability.

## Acknowledgments

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# REDUCING FEED-FOOD CONFLICT OF GRAIN-FED EUROPEAN FISHPONDS BY LOW-OPPORTUNITY-COST BIOMASS: PONDS, THE COW OF AQUACULTURE?

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**Introduction**

Every year, the Central Eastern European region fishponds (0.25 million hectares, 0.12 million tons fish) consume ≥0.3 million tons of grains such as wheat, corn, triticale, rye, and barley, that could be otherwise fed directly to human food chain. Pond-fed grains may occupy approximately 46000 hectares of arable land that could be directly used for cultivating human food. Although European fishponds have low footprints, this grain-feeding still poses feed-food conflict in the current food system and require alternatives. In the present experiment (vegetative season 2024), our aim was to understand the limits of European fishponds by reducing 55% of wheat input and replacing it with a low-cost, non-refined and plant-based LOCB (low-opportunity-cost-biomass, *i.e.* local agri-food by-products or co-products) feed that was fed in harmony with the pond's natural food web following the 'balanced pond feeding concept'. Specific objectives were to evaluate: (1) production performance, (2) environmental impact assessment (both LCA and *in-situ*), and (3) human food systems impact of reducing feed-food conflict associated with European fishponds.

**Materials and Methods**

The LOCB pond feed that was fed to common carp (*Cyprinus carpio*) resembled more of a ruminant/ monogastric feed (than a fish feed) having low crude protein (22.7%, lysine 0.9%, methionine 0.47%) possibly with high anti-nutritional factors (ANF), low crude fat (3.78%), and high fiber (neutral detergent fiber, NDF= 21%; acid detergent fiber, ADF= 14%; starch 27.7%) contents with poor feed conversion ratio (FCR ~4), measured in laboratory. It was composed of 71% human-inedible, locally-sourced LOCBs (breakfast-cereals factory discards, rapeseed, sunflower expellers, and corn DDGS) and 29% human edible biomass (28% wheat + 1% rapeseed oil) with formula cost of ~0.32 EUR $kg^{-1}$ near the cost of wheat (~0.20 EUR $kg^{-1}$). The LOCB pellet was twin-screw extruded floating-type. Wheat (fed as grains; sinking) was more refined, low protein (9.5%, lysine 0.27%, methionine 0.16%) without a high ANF, high in starch (59.1%), and low in fibers (NDF 9.9%, ADF 4.4%) with lab FCR also ~4. A set of 9 experimental fishponds (each 0.16 ha area, 0.8 m depth each, stocking density 471 kg scaly common carp $ha^{-1}$, initial body weight: 384 ± 81 g, 2+ years old) was assigned into two groups: "control" (triplicates) and "treatment" (six replicates). Treatment was nested (3+3) for optimizing LOCB feed utilization by fixed-time hand-feeding or all-day demand feeders. The isogenous stocked ponds received isogenous amounts of supplementary feed (3500 kg $ha^{-1}$) with control ponds fed traditionally with wheat. Total dosage was sub-divided into fixed monthly feeding plan: April (3%), May (7%), June (11%), July (32%), August (25%), September (15%), and October (7%). The treatment ponds received wheat during beginning-of-season (April to June, 22% of total input) and LOCB-feed during end-of-season (July to October, 78%). Ponds were fed daily. Monthly sampling for fish growth, water quality, plankton, and sediment (bi-monthly) were done. Ponds were harvested in beginning of November, filleting yield and fillet nutrient composition was assessed. Life cycle impact assessment and nutrient (material) flow analysis for food system was carried out.

**Results**

All ponds had statistically similar ($p>0.05$) survivability, fish yield, final fish size, FCR, filleting yield, EPA+DHA or total amino acids irrespective of control or treatment. Results suggest commercial production is not hampered by replacing 55% of wheat input with LOCB. Treatment carp was significantly ($p<0.05$) less fatty than control carp, which is a positive takeaway for regional fish gastronomy sector.

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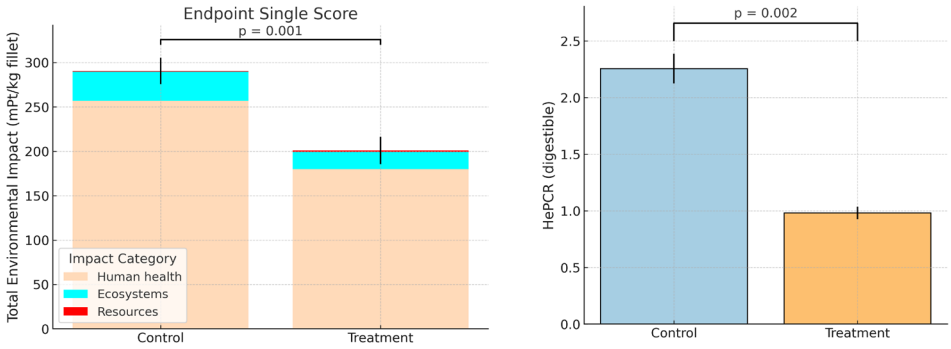


All ponds had statistically similar (*p>0.05*) chlorophyll-a, zooplankton trends, and nutrient (C, N, P) concentrations in water or sediment. The organic C reserves in pond soil before harvest were maximum in treatment ponds. Results suggest LOCB feeding cannot be associated with causing nutrient pollution or eutrophication.

In terms of food web, from a lab-measured FCR of ~4 (bad), both wheat and LOCB-feed showed pond-measured FCR of ~2.7 (good). The contribution from pond's natural food web to support carp yield was not altered (*p>0.05*) by replacing 55% of wheat input with LOCB. This is a win-win for fish farmers and water protection.

Total environmental impact was significantly lower (*p<0.01)* in treatment (200 mPt $kg^{-1}$ fillet) than control (290 mPt $kg^{-1}$ fillet). Impacts on human health and ecosystems are significantly lowered (*p<0.01*), except for resources consumption being significantly increased (*p<0.05*) due to capturing, mobilizing, and re-manufacturing LOCBs itself.

For the food system, the treatment became 'net producers of protein' with human edible protein conversion ratio (digestible), $HePCR_d$ of ≤1, significantly different (*p<0.01*) from control which was identified as net consumer of protein (HePCRd 2.2) due to input of human-edible wheat. Ponds are solely net-producers of n-3 LC-PUFA, as there is zero-input via wheat or LOCB-feed. The net n-3 LC-PUFA production is more sustainable (*p<0.01*) in treatment (500 mg $kg^{-1}$ human-edible ingredient) than control (250 mg $kg^{-1}$ human-edible ingredient). Results suggest a need for a reduction of feed-food conflict associated with grain-fed European fishponds, at least 55% as a starting point, by replacement with plant-based LOCBs.

**Discussions**

Integrating LOCB feeds would position European pond carp farming as the "cow of aquaculture and food systems" efficiently converting low-value biomass into nutritious food. Government subsidies should support such LOCB pond feed adoption, offsetting higher costs than grain-feeding to fulfill EU food system (*i.e.,* low feed-food conflict, net producers of farmed nutrients) and nature restoration targets (*i.e.,* higher recycling, regenerative farming). European fish-feed companies should seize the untapped market niche (≥0.3-million-ton $year^{-1}$) for LOCB-based pond feeds, uniquely suited for regenerative pond systems. Although gaseous emissions were not measured, sediment organic C reserves improved with LOCB feeds. Overall, at semi-intensive carp production levels (≤0.5-ton $ha^{-1}$ stocking, 1.2–1.4 tons $ha^{-1}$ yield in 7 months), LOCB feeds strongly outperform grain-based alternatives in reducing feed-food competition.

**Acknowledgment**: The study was funded by NAZV QK22010177 and OP JAK CZ.02.01.01/00/23_021/0012616 (PI: Jan Mraz). Thanks to Giovanni M. Turchini for awarding the metaphor: "Ponds – the holy cow of aquaculture".

# NEUROENDOCRINE STRESS RESPONSE TO STRESSORS OF DIFFERENT SEVERITY IN THE MUCOUSAL TISSUES OF S*parus aurata*

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## Introduction

It is known that fish are subjected to different stressors in the aquaculture industry such as anoxia or density. These stressors can activate a stress response, but the level of this response will depend on the severity of the stressor. Such severity may be due to different factors such as the species studied, the age, or duration, intensity and type of stressors among other factors. In this case it was selected a stressor of high severity such as anoxia where the fish must be out of the water, and a crowding stress which is less severe than anoxia. Moreover, crowding stress has an additional emotional component that anoxia does not have. Also, the stress response can modulate other responses such as the immune or the endocrine and energetic response that can also have further effects on fish health. This is why the aim of this study was to investigate the neuroendocrine stress response of a juveniles of *S.aurata* subjected to two different severe stressors such as anoxia and crowding to understand how the severity can modulate the activation level of the response. Also, another objective was to observe if the recovery time was affected by the severity of the stressor.

## Materials and methods

A total of 48 gilthead seabream juveniles were acclimated to AQUAB fish facilities (Universitat Autònoma de Barcelona, UAB), After the acclimation period, fish were randomly divided in 3 different treatment groups; control, anoxia, and crowding group that were sampled at 1 and 24 h after the stressor. The fish subjected to anoxia were exposed to air for 3 min and then were placed back in thanks for 1 hour or 24 hours until the sampling. The crowding stress was performed by decreasing the water level from 20 L to 10 L, remaining like this for 30 min and then restoring the water volume, and wait until sampling at 1 or 24 hours. From each fish, plasma was sampled for haematological and biochemical analysis, and the mucosal tissues (gills, skin, and gut) were sampled for qPCR gene expression analysis.

## Results

No differences were found in cortisol levels in plasma, but the parameters related with the oxidative stress changed in the groups subjected to stress compared with controls. Regarding the gene expression results, it was observed a tissue-specific response, being the gills the most affected one. What was relevant is that glucocorticoids receptors did not show any difference compared with the control, but in the case of *cfos* gene there was an upregulation that can be clearly associated to the stress response. Moreover, the genes related with the immune and the oxidative stress were also affected. Another interesting discovery was that crowding stress showed more effects than anoxia, and the effects from the density lasted for longer time in the fish.

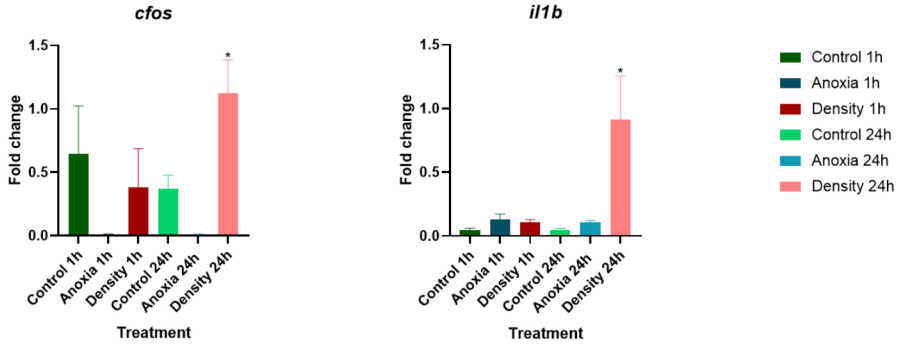


Figure 1. Fold change of a) cfos and b) il1b of the gill after the stressors tratments. The * symbol indicates the group significantly different from the others.

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**Discussion**

As it was expected different severities had different effects on the fish (Figure 1), since anoxia did not cause the upregulation of *cfos*, but the crowding stress caused an upregulation but after 24 hours. Despite glucocorticoids receptors were not significantly regulated, *il1β* in the case of the crowding group at 24h suffered an upregulation and an interesting fact is that the anoxia exposure did not affect the immune genes in gills. Also it is relevant to remark the tissue specific response, as this could be due to the exposure of each tissue to the stressors and the main function of the tissue. In the case of gills and the skin both tissues are in direct contact with the environment but the main specific function of each tissue is completely different which may explain the different sensitivity of the response

# USE OF A BLEND OF WHITE GRAPE MARC EXTRACT COMBINED WITH MICROALGAE FOR DIETARY FORTIFICATION IN JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

In recent decades, the search for functional ingredients to overcome the effects of reducing the use of animal marine protein in aquafeeds has been one of the great challenges in the aquaculture sector. In response to this problem, terrestrial plant feedstuffs have emerged as possible alternative proteins to fishmeal (Quagliardi *et al.,* 2024). In spite they have a high protein content, they may contain anti-nutritional compounds that can induce digestive problems in animals and hinder the nutrient digestibility (Seibel *et al.,* 2022). In this sense, the concept of dietary fortification arises as a feeds supplemented in very low proportions with functional ingredient that can promote fish growth, health and improve the resistance of animals to diseases and stress, even improving the digestibility of plant-based aquafeeds. From a nutritional point of view, algae are a natural source of proteins, lipids, vitamins and carotenoids, whose use is associated with beneficial effects on fish status. Similarly, white grape marc extract contains polyphenols which could help to mitigate the potential negative effects they might cause in fish (Quagliardi *et al.,* 2024). Thus, the main objective of this study was to evaluate the effect of dietary fortification with a functional ingredient based on white grape marc extract and microalgae, in liquid and solid format, in feeds with a high proportion of plant protein ingredient on growth performance, proximal muscle composition and digestive functionality in juvenile rainbow trout (*Oncorhynchus mykiss*).

## Material and Methods

The feeding trial was carried out at the Aguas Blancas Fish Farm (Quéntar, Granada). For this purpose, three experimental diets were formulated: a control diet that did not contain the functional ingredient (CT) mimicking the formulation used in commercial aquafeeds, and two diets similar to the previous one, but 2% fortified with the functional ingredient in liquid (NL) and solid (NS) format. Fish were fed for 100 days until triplication of their initial body weight (12.5 g). At the end of the feeding trial, growth performance and zootechnical parameters were evaluated, and samples of muscle tissue were obtained for analysis of proximal composition, and intestine for evaluation of pancreatic and intestinal digestive enzyme activities.

## Results and Discussion

The final weight of fish fed the diet fortified with NL (57.9 ± 12.2 g) was higher than that of fish fed CT (54.7 ± 12.3 g) or NS (54.0 ± 12.8 g) diets. However, body length did not differ among experimental groups. Regarding the composition of muscle, lipid content decreased in specimens of NL and NS groups compared to CT group, which might indicate a better utilization of lipid as source of energy in these groups (Perera *et al.,* 2020).

Figure 1 shows digestive enzymatic activities measured in fish fed the different dietary treatments. Overall, higher trypsin and chymotrypsin activities were observed in the NS group, which could be associated with better digestion processes at intestinal level. However, leucine aminopeptidase and alkaline phosphatase activities were higher in fish of NL group compared to specimens from the NS batch. The observed changes in the digestive enzymatic activities seems to reflects that dietary fortification with this functional ingredient can be useful for modulating the digestive functionality in juvenile rainbow trout. Further analysis are in progress for ascertaining the effect of these fortified diets on the intestinal microbiota and the antioxidant capacity in these fish.

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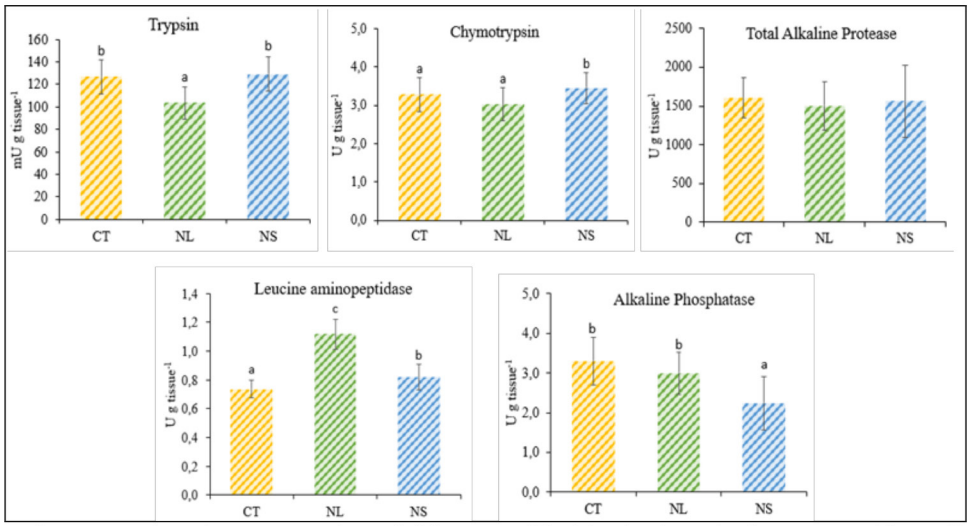


**Figure 1.** Digestive enzymatic activities quantified in the intestinal extracts of juvenile *O. mykiss*.

**Acknowledgements**

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# PHYSIOLOGICAL RESPONSES OF TURBO SAZAE TO CLIMATE-DRIVEN DIETARY SHIFTS IN BENTHIC ECOSYSTEMS AROUND JEJU ISLAND, REPUBLIC OF KOREA

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**Introduction**
Ocean warming due to climate change has significantly altered the distribution and physiology of marine species. Jeju Island, located in rapidly warming waters, has seen a decline in kelp beds and a rise in coralline algae, affecting local biodiversity and food webs. *Turbo sazae*, a subtropical gastropod, is influenced by these habitat changes and food availability. Understanding its physiological response to dietary shifts is critical. This study investigates how different macroalgal diets affect *T. sazae* under controlled conditions.

**Materials and Methods**
Ninety *T. sazae* specimens were collected and acclimated before being divided into groups fed with either *Ecklonia cava* or *Corallina officinalis*. The experiment lasted 18 weeks in controlled tanks at 20°C with weekly feeding. Wet weight, immune function, gonad development, and biochemical composition were analyzed. Hemocyte parameters were measured using flow cytometry, and tissues were used for proximate and amino acid profiling. Statistical analyses were conducted using ANOVA and t-tests.

**Results**
throughout the 18-week experiment, *T. sazae* showed no significant differences in wet weight between groups fed with *E. cava* or *C. officinalis*, indicating both diets were nutritionally adequate. Hemocyte parameters including viability, phagocytosis, and ROS production remained stable and showed no significant variation between feeding groups or over time. Gonad development progressed similarly in both groups, with most individuals remaining in the ripe stage and limited spawning observed, likely due to the constant 20 °C water temperature. Proximate composition analysis revealed decreases in carbohydrate content and increases in lipid content over time, while protein levels remained consistently high. Amino acid profiling showed significant temporal and feed-related changes: glutamic acid, taurine, and aspartic acid declined initially, but later increased in the *C. officinalis*-fed group, suggesting enhanced metabolic activity; in contrast, *E. cava*-fed individuals showed decreased glycine and proline levels. These results indicate diet-specific influences on amino acid composition and energy metabolism, despite overall physiological stability.

**Discussion**
*T. sazae* exhibited dietary adaptability, with both macroalgae supporting immune function, gonad development, and biochemical stability. Lipid accumulation and AA fluctuations reflect metabolic adjustments to environmental stress. Although total protein was unaffected, feed type influenced AA composition, which may impact tissue function. Future studies should assess long-term effects under natural environmental variability.

**Conclusion**
*T. sazae* maintained physiological stability under both feeding conditions, demonstrating adaptability to dietary changes. While no major differences in growth or immunity were observed, AA composition varied with diet, suggesting subtle metabolic effects. Further field research is needed to evaluate the ecological implications under climate change.

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# IMPACT OF TRANSPORT ON *Laminaria ochroleuca* SEEDLINGS FOR SEAWEED FARMING

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## Introduction

The transportation of seaweeds from the hatcheries to the cultivation sites represents a critical step in aquaculture, with direct implications on the physiological integrity and subsequent survival of the seedlings. Transport usually occurs without immersion in seawater, using cool, humid conditions maintained by isothermal containers to reduce desiccation and thermal stress. Controlled temperature and humidity are essential, as even short-term exposures to suboptimal conditions can cause physiological deterioration and reduced viability. These risks are particularly relevant when hatcheries are located far from offshore cultivation sites, making transport logistics a key factor for successful farming.

*Laminaria ochroleuca*, has attracted growing interest in Portugal due to its ecological adaptability and commercial value in the food, cosmetics, and biotechnology sectors. As a native species with promising biochemical traits, it is considered a strong candidate for large-scale aquaculture. Successful cultivation depends on maintaining seedling quality throughout the production cycle, including during transportation. Stress during this stage can impair photosynthesis, alter pigmentation, and reduce post-deployment performance.

This study aimed to assess the impact of transportation on the physiological condition and survival rate of *L. ochroleuca* seedlings before deployment at sea, to support the development of evidence-based protocols that ensure viability and optimize offshore yields.

## Material and Methods

To simulate the transport conditions of *L. ochroleuca* seedlings, isothermal containers with an ice pack were used. Seeded spools were placed inside the containers without seawater, under high relative humidity, reproducing the environmental conditions experienced during transport from the hatchery to the deployment site. Temperature and relative humidity were continuously monitored using appropriate sensors during experiment period.

Physiological condition of seedlings was assessed at 0, 6, 12, and 24 hours after the beginning of the simulation trial. At each time point, the maximum quantum yield of photosystem II (Fv/Fm) was measured using a Junior-PAM fluorometer, as a proxy for photosynthetic efficiency and overall physiological status. Photographic documentation of the seedlings was also performed at each time point to detect any visible colour changes that could indicate stress-related responses.

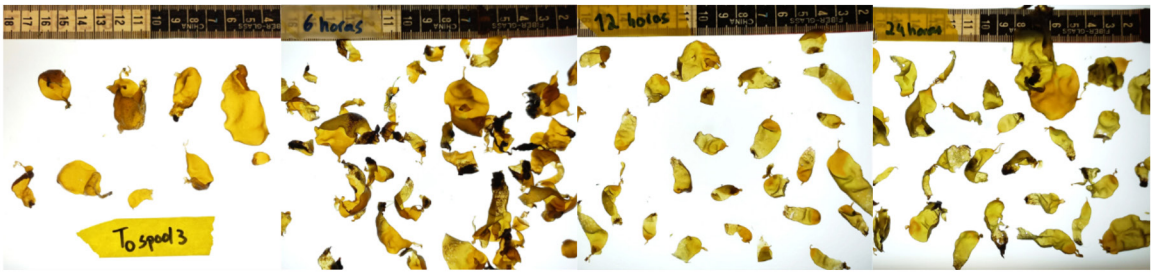


Figure 1. Images of *L. ochroleuca* seedlings after being transported in isothermal containers for 0, 6, 12, and 24 hours

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Following the respective transport period, seedlings were transferred back to a controlled culture system for a period of one week. The culture tanks were maintained under standard cultivation conditions (14ºC and 12h:12h). At the end of this period, Fv/Fm measurements and photographic assessments were repeated to evaluate the potential of recovery of physiological condition and viability of the seedlings.

**Results and Discussion**

During the first 6 h the Fv/Fm remained stable and similar to that observed in the beginning of the trial ($0.77 \pm 0.04 – 0.75 \pm 0.06$, $p \leq 0.05$). However, these values significantly decreased after 12h ($0.56 \pm 0.3$) and 24h ($0.35 \pm 0.3$). Indeed, the proportion of individuals in good physiological condition (Fv/Fm > 0.65) at the beginning of the trial (97%), was significantly reduced to 63% and 43% after 12h and 24 h, respectively. On the other hand, the proportion of individual considered non-viable (Fv/Fm < 0.2) raised to 23% and 43% during the same period.

The negative effect of transportation was also reflected in the coloration of *L. ochroleuca*. Seedlings exhibited a typical bright brown coloration at the beginning of the trial, which gradually shifted to a greenish coloration after 6h–24h.

After one week of "recovery" in standard culture conditions, Fv/Fm results showed that the proportion of individuals in good physiological condition further declined to 33% and 13% in the 12h and 24h transportation treatments, respectively.

The results revealed that *L. ochroleuca* seedlings maintain stable physiological performance during the first 6 hours of simulated transport, as evidenced by consistent Fv/Fm values and minimal visual changes. However, extended exposure (12–24 hours) led to a significant decline in photosynthetic efficiency and an increase in the proportion of non-viable individuals. The shift in coloration from bright brown to greenish tones further supports the onset of physiological stress during prolonged periods under transport-like conditions.

Recovery after one week in standard culture conditions was limited, particularly for seedlings exposed to 12 and 24 hours of simulation, where the proportion of physiologically healthy individuals remained low. These results suggest that the effects of extended transport are not easily reversible and may compromise seedling quality. Therefore, under the investigated conditions, transport duration should ideally not exceed 6 hours to preserve physiological integrity and maximize the success of subsequent deployment. Nonetheless, even after 12 hours, 33% of the individuals remained in good physiological condition, which may still be acceptable for achieving effective coverage in high-density deployments.

**Acknowledgments**

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# UNDERSTANDING FISH BEHAVIOR FOR IMPROVED AQUACULTURE FEEDING MANAGEMENT

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Feed inefficiency remains a critical challenge in global aquaculture, with Norway alone wasting up to 10\% of annual feed inputs [1].

Our study developed a comprehensive monitoring framework integrating 3D multibeam sonar technology [10] with environmental sensing to decode fish behavior patterns in sea cages near Bergen, Norway (Figure 1). The system captured continuous volumetric data while monitoring key environmental parameters including temperature, oxygen levels, and current velocity [8].

Our methodology employed Maximum Biomass Depth (MBD) calculations and Boundary Biomass (BB) detection [3] to track fish aggregations, enhanced through k-means clustering [7]. This revealed consistent diurnal patterns (Figure 2), with fish aggregating deeper (10-20 m) at night [12] and moving toward surface waters (3-10 m) during daylight. These patterns showed strong alignment with feeding times [6], suggesting anticipatory behavior.




*Fig. 1: Bergadalen aquaculture site (60.121 ◦N, 5.908 ◦N) on the west coast of Norway. The thick red arrow shows the position of the SeapiX sonar on the side of the test cage. Thin arrows indicate the x- and y-axes. The thick light blue arrow marks the location of environmental instruments at 5 m and 15 m depths*

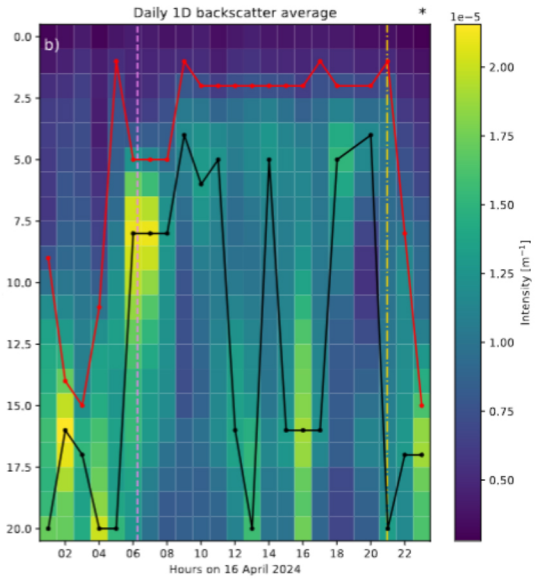


*Fig. 2: Hourly averaged intensities in the yz-direction from 16 April 2024 over the whole day. The red line represents a boundary biomass detector where underneath fish accumulate. The black line represents maximum biomass depth values, where fish accumulate each hour. The star (*) marks 23:00 on 16 April 2024.*

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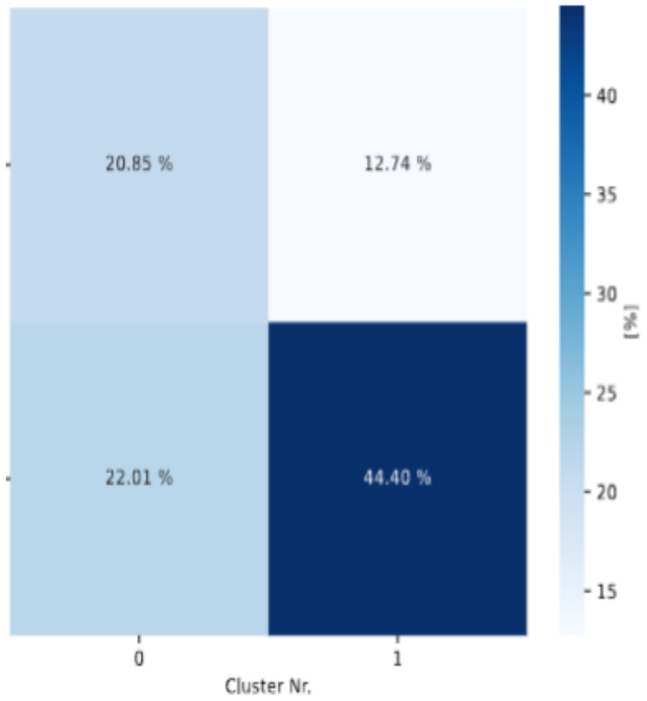


*Fig. 3: Based on cluster results, the distribution of maximum, biomass depth values is split between 3 - 10 m and 10 - 20 m.*

Vertical distribution analysis quantified behavioral states, showing fish spend 66.4% of low-activity periods at 10-20 m depths [11] while distributing more evenly during high-activity feeding periods. These findings enable data-driven feeding optimization [5] while improving fish welfare [4].

The study demonstrates how advanced monitoring can transform aquaculture practices [2]. Future work will focus on real-time implementation [9] to enhance feeding efficiency and sustainability.

**Acknowledgements**
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# BETTER STUNNING, BETTER QUALITY: IMPACTS OF STUNNING METHOD ON BRAIN FUNCTION AND PRODUCT QUALITY IN TILAPIA (*Oreochromis niloticus*) AND CATFISH (*Clarias gariepinus*)

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**Background**
With increasing societal and regulatory focus on ethical practices in aquaculture, the identification of humane stunning and killing methods has become imperative. Electrical and percussive stunning techniques have been proposed as viable means to minimize suffering by ensuring rapid and sustained loss of sensibility. However, for species extensively farmed for human consumption, such as Nile tilapia (*Oreochromis niloticus*) and African sharptooth catfish (*Clarias gariepinus*), it is critical to evaluate stunning methods not only in terms of welfare but also regarding their effects on product quality. A multidisciplinary approach, combining assessments of brain function with product quality, provides critical insights into the broader implications of stunning practices.

**Material and methods**
Electrical stunning was performed using an in-air electrical stunner prototype (Optimar AS, Norway) delivering a 20 s stun at maximum current of ~3.9 A for tilapia and ~5.7 A for catfish. Percussive stunning was carried out with an automated percussive stunner (Baader, Germany), set to deliver ~60 J (velocity of ~28 $ms^{-1}$) and ~71 J (velocity of ~31 $ms^{-1}$), for tilapia, while catfish were exposed only to the higher settings.

Immediately post-stunning, electroencephalogram (EEG) electrodes were attached to record brain function continuously for 10 min. Stunning effectiveness was determined by the presence or absence of visually evoked responses (VERs) in the EEG recordings. All groups assessed for VERs are presented in Figure 1. Product quality assessments were conducted only on groups subjected to maximum stunning intensities, evaluating water holding capacity and rigor mortis progression. The water-holding capacity of flesh was measured as drip loss (%) which was determined as percentage weight loss relative to initial fillet weight at 4, 24, 48, 72, and 96 hours post-stunning (n = 8 per group). Rigor mortis was monitored hourly over 7 h post-stunning, quantified via a rigor index (%) based on vertical displacement of the caudal fin (n = 6 per group).

**Results**
At the lower electrical stunning intensities, 25-100% of tilapia and 100% of catfish recovered during monitoring, whereas at maximum intensity, recovery decreased to 20% in tilapia and 67% in catfish (Fig. 1). For tilapia, percussive stunning at ~60 J was insufficient to ensure insensibility in all individuals; however, ~71 J achieved a 100% success rate. In catfish, one recovery occurred after percussive stunning (~71 J), attributed to a misaligned strike targeting the nose rather than the brain. Preliminary analyses indicated that drip loss increased more rapidly over time in electrically stunned tilapia compared to percussively stunned individuals (Time x Treatment: $F_{2.0,28.5} = 13.7$, $p < 0.001$). Also in catfish, drip loss was significantly higher in electrically stunned individuals compared to those stunned percussively (Treatment: $F_{1.0,14.0} = 5.1$, $p = 0.04$). However, the difference in drip loss between groups remained consistent over time (Time x Treatment: $F_{1.1,15.6} = 2.6$, $p = 0.124$). The onset of rigor mortis typically occurred between 1 and 4 h post-stunning across methods. However, after 7 h, electrically stunned tilapia showed a significantly higher rigor index (87.0 ± 8.3%) than percussively stunned fish (22.4 ± 5.8%, $t_9 = -6.7$, $p < 0.001$), while no differences were observed in catfish (7.9 ± 4.2% *vs*. 12.1 ± 2.5%, $t_{10.0} = 0.9$, $p = 0.205$).

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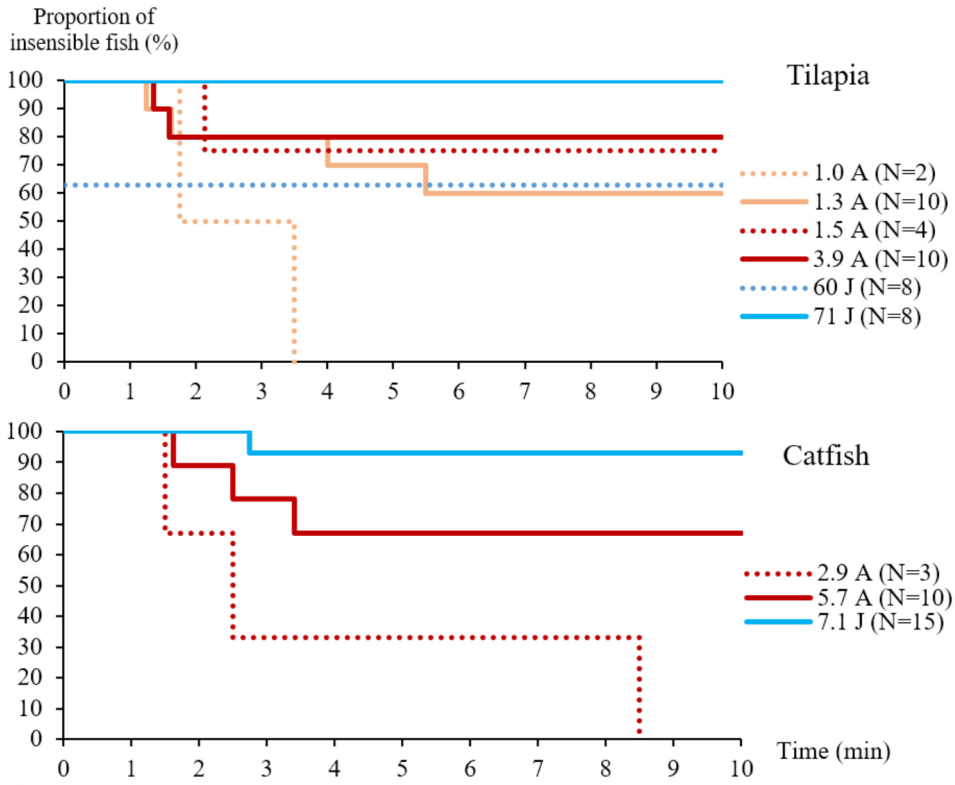


**Figure 1. The proportion of insensible tilapia and catfish during the monitoring period following electrical and percussive stunning.** Kaplan-Meier distributions of electrically (orange and red dashed and solid lines) and percussively (blue dashed and solid lines) stunned tilapia (upper panel) and catfish (lower panel).

## Conclusions

In both tilapia and catfish, a proportion of individuals regained brain function following electrical stunning. Although increasing stun intensity improved stunning efficacy, this was associated with compromised product quality, increasing drip loss and accelerating the development of rigor mortis, thus having a negative impact on water holding capacity, processing, and texture of the flesh. Automatic percussive stunning achieved higher welfare outcomes when appropriate force and accuracy were applied and better preserved product quality. Our findings demonstrate that percussive stunning may be a commercially viable solution for tilapia and catfish, aligning both welfare and quality objectives. Further research should explore the consistency of force and accuracy under large-scale commercial production conditions.

# HATCHERY PRODUCTION OF THE STRIPED VENUS CLAM *Chamelea gallina* FOR FISHERY RESTORATION, AND GENETIC VARIABILITY IN THE SEED

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## Introduction

The striped Venus clam is an infaunal, subtidal species living in the Mediterranean Sea and its neighbour Atlantic coasts, and in the Black Sea. The species is exploited as a fishery but is facing a reduction in catches. In the Gulf of Valencia (Spain) and neighbouring areas, the fishery has collapsed and has been closed, but in spite of the ban, it has not recovered (Baeta, 2021). Supplementation of wild populations with clam seed obtained in captivity by using bivalve aquaculture methodology might help the recovery of populations and favour an aquaculture industry. A problem with this strategy is that the high fecundity and large variance of family size in bivalves may lead to large numbers of spat that have less genetic variability and higher relatedness than the supplemented population. Therefore, a strict control of the parental contributions and the genetic variability in the offspring is recommended. Here, the successful production of striped Venus clam spat in hatchery under controlled conditions, and the characterization of its genetic variability, are reported.

## Materials and Methods

Clams (N = 350) were collected near Gandía (Valencia, Spain) in May 2023, before the peak of the reproductive season (summer), in order to have as much control as possible of the gonad maturation process. They were placed in 150L PVC tanks with running seawater, and were subjected to two conditioning treatments, with two replicates each: food only (FO) and food plus temperature (FT, average 2°C above ambient). Food ration was 3% of average clam dry meat weight, with 50% *Isochrysis glabana*, 30% *Chaetoceros calcitrans*, and 20% *Tetraselmis suecica*. Gonad maturation was studied by histology and condition indices (CI). The gametogenic stages were classified as in Delgado et al. (2013). A CI = dry meat weigh / dry shell weight was used. After 4-5 weeks of conditioning, the broodstock was subjected to thermal shocks (17-27°C ) to induce spawning. Larvae were cultured in 200 L conical tanks. Water was changed three times a week. When most larvae had reached the pediveliger stage, they were moved to 200 µm mesh-bottom containers for settlement. Larvae and postlarvae were fed *Isochrysis galbana* ($10^5$ cells/ml).

The 11 parents and 72 spat were genotyped for 11 microsatellites with the primers designed by Papetti et al. (2016). Allele frequencies and gene diversity were calculated with the program Arlequin (Excoffier et al., 2025).

## Results

Histology and condition indices of the broodstock showed that most clams were sexually mature after conditioning with the FO treatment, while before conditioning most animals were immature. The 17-27°C thermal shocks induced spawning only in clams from the FO treatment. Six females and five males spawned, and three million eggs were recovered. *Ca*. two million larvae were alive at 48 hours after fertilization. The size of the larvae increased up to an average of 220 µm and reached a plateau 21 days after fertilization. Survival at this point was 25%. Larvae were then moved to the settlement tanks. A few days later water temperature raised suddenly to 29°C, coinciding with a summer heat wave, and mass mortality of the larvae occurred. Surviving spat were collected for further study.

All scored microsatellites were polymorphic. Out of the 57 alleles observed in the parents, 49 were present in the offspring, and nine new alleles appeared by mutation. Gene diversity in the parents was 0.624±0.040 and 0.554±0.007 in the progeny.

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## Discussion

Rearing larvae obtained from spontaneous spawning in captivity of clams collected at the peak of the reproductive season was achieved by Joaquim et al. (2016). However, this approach provided no control over the parental contribution to the offspring. These authors also tried inducing spawning in controlled conditions by thermal shock without success. Here, it has been shown that conditioning of clams collected before the natural reproduction peak during four weeks at ambient temperature results in large numbers of larvae, and potentially large numbers of spat. However, too high a seawater temperature can be a problem, inducing spontaneous spawning before the gonad is completely mature, and mortality during the larval to juvenile metamorphosis stage.

The genetic characterization of the parents and the progeny showed that 14% of the parental alleles and 11% of gene diversity were lost due to due to the variable contribution to offspring among parents. Clearly much larger numbers of parents are needed to produce clam seed that meets the recommended standards for supplementation of wild populations. Prolonging the conditioning period, increasing the food ration and changing the diet composition are three factors that should be explored to achieve this goal.

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# EXPLORING THE EFFECTS OF BIOFLOC TECHNOLOGY ON GROWTH PERFORMANCES OF TEMPERATE FRESHWATER FISH AND HOW FISH SPECIES MAY INFLUENCE MICROBIAL DYNAMICS

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## Introduction

Biofloc technology (BFT) offers new opportunities for intensive freshwater aquaculture by improving water quality through microbial flocculation and nutrient recycling[1]. It also allows for reduced water consumption, better feed conversion, and the potential reuse of microbial biomass as a nutritional resource. While widely used for tropical species like tilapia (*O. niloticus*) and shrimp, its application to temperate freshwater fish is limited and relatively unknow. The POLYRAS project (Interreg GR) aims to diversify and develop polyculture, which is widely used in pond systems, in three sustainable aquaculture systems: Recirculating Aquaculture Systems (RAS), aquaponics systems, and BFT. Within this cooperative project, our study focuses specifically on the Biofloc technology.

This work explores the possibility to rear new temperate freshwater species in polyculture in BFT mainly focusing on survival rate and growth performances. The six selected species, black bass (*Micropterus salmoides*), Russian sturgeon (*Acipenser gueldenstaedtii*), pikeperch (*Sander lucioperca*), perch (*Perca fluviatilis*), catfish (*Silurus glanis*), and tench (*Tinca tinca*), were chosen based on their economic potential in the Greater Region and adaptation to different polyculture scenarios, as identified in the TOFF database[2]. As most of these species have not been previously reared in BFT systems, this preliminary study was designed to screen and identify new candidate species for BFT aquaculture, evaluating their growth and investigating how each species may influence microbial dynamics in the system.

## Material and Methods

The experiment was performed in three individual 250 L tanks maintained in BFT and one 250 L control tank in clear water (RAS). Fish were stocked at an initial density of 6 kg/m³, with the number of individuals adjusted according to species size. Each species was successively reared for a period of three weeks and fed to satiation throughout the experimental period.

Growth performances were assessed through morphometric measurements (body weight and total or fork length) and food conversion ratio (FCR) assessment. Water quality was monitored daily (t°, $O_2$, floc volume) or twice a week (pH, conductivity, TAN, and alkalinity) to ensure stable environmental conditions.

At the end of the experiment, water samples from BFT tanks were analyzed to characterize microbial communities. Zooplankton and phytoplankton were counted in 1 mL subsamples, and floc particle size and bacterial density were measured.

## Results and discussion

The performance of black bass in BFT is promising, with 100% survival, as in the RAS control, and a positive growth (3 g), slightly lower than in RAS (5.9 g). In contrast, sturgeon showed variable survival (50–100%) and marked weight loss in BFT (−16.5 to −61.4 g), while 100% survival and significant weight gain (44.3 g) were recorded in RAS. With stable water quality parameters, no specific issue could explain these results, suggesting sturgeon may not be suitable for BFT systems. Preliminary trials with perch and catfish were stopped after one week due to signs of stress, such as reduced feeding and abnormal behavior. These findings highlight both the potential and the limitations of BFT for temperate freshwater species (Table 1).

Pikeperch demonstrated good growth performance across all BFT tanks (9.3 g). Survival was affected by elevated ammonia concentrations linked to a temporary instability of the biofloc. Under more stable conditions, pikeperch could be successfully reared in BFT. Tench demonstrated excellent adaptability to BFT system, with 100% survival (Table 1). Individual weight gain ranged from 34.7 to 51.1 g in the BFT tanks, slightly surpassing the 34.5 g average in the RAS control. The stability of physicochemical parameters throughout the trial supported these good performances (Table 1).

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*Table 1 : Summary of survival, growth, and feed efficiency data reared for three weeks in BFT (mean of 3 tanks) and RAS.*

| | Black-bass | | Sturgeon | | Pikeperch | | Tench | |
|---|---|---|---|---|---|---|---|---|
| | BFT | RAS | BFT | RAS | BFT | RAS | BFT | RAS |
| Survival (%) | 100 | 100 | 72 | 100 | 77 | 100 | 100 | 100 |
| N initial | 36 | 37 | 6 | 5 | 19 | 19 | 6 | 6 |
| Initial mean weight (g) | 42.0 | 41.5 | 274.4 | 317.6 | 77.7 | 79.4 | 245.1 | 252.7 |
| Individual weight gain (g) | 3.0* | 5.9 | -39.7 | 44.3 | 9.3 | 21.5 | 42.5 | 34.5 |
| FCR | 2.9* | 1.8 | / | 1.2 | 1.2* | 0.7 | 1.9 | 2.3 |

(*: Data based on two tanks for Black-bass and one tank for Pikeperch)

*Table 2 : Mean (±sd) abundance of rotifers, ciliates and phytoplankton (ind./mL) in BFT water at the end of the rearing period for each species.*

| | Black-bass | Sturgeon | Sander | Tench |
|---|---|---|---|---|
| N rotifers/ml | 4 ± 4 | 12 ± 16 | 6.7 ± 11 | 10 ± 5 |
| N ciliates/ml | 73 ± 43 | 214 ± 137 | 1577 ± 1714 | 1799 ± 1978 |
| N phytoplankton/ml | 44 ± 28 | 36 ± 45 | 5 ± 6 | 13 ± 10 |

The analysis of planktonic communities suggests that species reared in BFT systems may influence the abundance and composition of phytoplankton and zooplankton, with noticeable differences in ciliate, and phytoplankton counts (Table 2). Measurements of floc particle size and bacterial abundance are still in progress and should further clarify the interactions between fish species and microbial dynamics in BFT systems.

These preliminary results highlight black bass, pikeperch and tench as promising candidates for BFT culture.

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# IMPACT OF INCREASING TEMPERATURES ON NEUROENDOCRINE AND MOLECULAR RESPONSES OF SKELETAL MUSCLE AND LIVER IN FISH

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Global climate change is driving a continuous rise in water temperature, posing significant challenges to aquaculture productivity, animal welfare, and product quality. As ectothermic organisms, fish rely on ambient temperature to regulate metabolic and physiological processes, rendering them particularly vulnerable to thermal stress (Ahmad et al., 2011). This review synthesizes current knowledge on neuroendocrine and molecular mechanisms underlying the responses of skeletal muscle and liver—two critical tissues for growth and energy homeostasis—under elevated temperature conditions.

Thermal stress triggers activation of the hypothalamic–pituitary–interrenal (HPI) axis and the brain–sympathetic–chromaffin cell pathway, leading to cortisol and catecholamine release. These endocrine mediators mobilize energy reserves, modulate carbohydrate and lipid metabolism, and regulate immune and oxidative stress responses (Alfonso et al., 2021). In skeletal muscle, increased temperature induces the expression of heat shock proteins (Hsp70, Hsp90), which safeguard protein folding and stability, while also affecting growth-regulating pathways such as growth hormone (GH) and insulin-like growth factors (IGF). Transcriptional regulation of myogenic factors (MyoD, Myf5, myogenin) and transforming growth factor-beta family members (myostatin, follistatin) influences myofiber hypertrophy and hyperplasia, ultimately shaping muscle growth potential (Akbarzadeh et al., 2018).

The liver exhibits profound transcriptomic and proteomic changes under heat stress, including modulation of antioxidant defenses (Sod, Cat, Gpx), metabolic pathways, immune signaling cascades (NF-κB, IL-17), and apoptosis regulators (Aidos et al., 2020). Proteomic analyses further highlight alterations in proteins involved in cellular homeostasis, energy production, and cytoskeletal integrity. Additionally, specific microRNAs (e.g., miR-122, miR-194-3p) emerge as important regulators of glycolysis and lipid biosynthesis under high-temperature regimes, providing potential molecular biomarkers for stress adaptation (Mohanty et al., 2018).

While some of these responses are adaptive in the short term, chronic exposure to elevated temperatures can lead to growth suppression, reduced feed conversion efficiency, impaired immunity, and increased susceptibility to disease. In the context of aquaculture, these effects translate into lower yields, higher production costs, and compromised sustainability. Mitigation strategies include selective breeding for thermal tolerance, nutritional interventions targeting oxidative stress and growth pathways, and technological improvements in farming systems to optimize environmental control.

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This comprehensive understanding of neuroendocrine and molecular responses to heat stress is essential for developing climate-resilient aquaculture practices. By identifying key biomarkers and regulatory mechanisms, the industry can improve the capacity of farmed fish to cope with thermal challenges, ensuring long-term productivity and food security.

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# PUBLIC ATTITUDES TOWARDS AQUATIC ANIMAL WELFARE

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## Introduction

Each year, up to one billion fish are farmed in the European Union [1]. Despite this scale, there is currently no species-specific legislation that addresses their distinct welfare needs. Consequently, poor farming conditions, including overcrowding, disease, and inhumane slaughter methods, remain common. A growing body of scientific evidence affirms fish sentience, yet welfare protections lag behind those for terrestrial farmed animals.

## Objectives

This study aimed to assess public attitudes toward aquatic animal welfare across the EU, evaluate awareness of fish farming practices, and understand how these perceptions influence consumer behaviour.

## Methods

The survey [2], commissioned by Compassion in World Farming and Eurogroup for Animals, was conducted by Sapience between 20 March and 5 April 2024. A total of 9,225 participants from nine EU countries—Czechia, France, Germany, Greece, Italy, Poland, Spain, Sweden, and The Netherlands—completed an online questionnaire. Questions addressed attitudes toward fish sentience, awareness of farming methods, support for legislative change, and consumer preferences for welfare-friendly products.

## Results

The findings reveal strong public concern for fish welfare, with most supporting stronger legislative protections and demonstrating a preference for purchase higher welfare products:

- **91%** of respondents believe that fish welfare should be protected to the same or a greater extent as other farmed animals.
- **4 in 5** support legislation requiring best practices based on scientific evidence to meet fish welfare needs.
- **71%** agreed that fish can feel pain, with the highest agreement in Czechia (**77%**).
- **A significant majority of people in Spain (65%) consume fish at least once a week**. However, awareness of current fish farming methods was low: in Spain, **63%** reported low awareness, the lowest of all countries surveyed despite their high fish consumption—a significant majority of people in Spain (65%) consume fish at least once a week, which is notably higher than the overall EU average of 40%.
- Only **39%** knew that most farmed fish are not stunned before slaughter, but **70%** supported making stunning a legal requirement.
- **90%** expressed a preference for purchasing fish raised in higher welfare conditions. In Germany, **74%** supported clear welfare labelling for fish products.

## Conclusion

The survey results highlight significant public support across the EU for improving the welfare of farmed aquatic animals. Awareness of farming practices remains limited, yet consumer demand for higher welfare products is strong. These findings underscore the urgent need for legislative action to close the welfare gap between aquatic and terrestrial farmed animals.

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# EFFECTS OF DIFFERENT LEVELS OF FISHMEAL AND FISH OIL REPLACEMENT WITH ALTERNATIVE PLANT PROTEIN AND LIPID SOURCES IN AQUAFEEDS FOR FLATHEAD GREY MULLET (*Mugil cephalus*) JUVENILES

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## Introduction

The development of sustainable aquaculture relies on diversifying production with low-trophic-level species whose nutritional needs do not depend on fishmeal (FM) and fish oil (FO). Given that feed costs represent approximately 60% of aquaculture production expenses (APROMAR, 2022), farming species with reduced FM and FO diets offers both environmental and economic advantages. Flathead grey mullet (*Mugil cephalus)* is a promising candidate due to its natural lack of dependency on FM and FO (Gisbert et al., 2016; Wassef et al., 2002)optimizing weaning diets is of special importance for this species at this particular stage of development. Thus, authors have tested a weaning protocol for wild flathead grey mullet fry (202mg initial body weight. However, despite extensive knowledge of its natural feeding habits (Brusle, 1981), data on its nutritional requirements and response to formulated feeds remain limited. Moreover, the effects of these low FM and FO diets on the polyunsaturated fatty acids (PUFA) content of the fish must be taken into account, as this is an important factor in the quality of the final product (Sprague et al., 2016)as an oily fish, contain a rich source of the health promoting long-chain omega-3 fatty acids, eicosapentaenoic (EPA. Scientific research is therefore essential to know the appropriate composition of the specific diet for this species and the identification of the best culture methods.

## Material and Methods

Two feeding trials were conducted using juvenile flathead grey mullet hatched and reared at IRTA. In Trial 1, 495 fish (11.77 ± 2.17 g) were randomly distributed into nine 500 L tanks (IRTAmar® water recirculation system) to test three diets (triplicate groups): Control (20% FM), FM-0 (0% FM), and FM-5 (5% FM). After 72 days of *ad-libitum*-feeding trial, a final biometric sampling was performed, and samples of plasma, liver, and muscle were collected. Somatic and zootechnical indices were calculated, and samples from each tissue were analysed (biochemical profile in plasma and circulating cortisol levels; proximate composition and fatty acid profile in liver and muscle; and gene expression in liver). Trial 2 required 720 fish (16.39 ± 4.07 g) distributed across 18 tanks (200 L) in two salinity conditions (36 and 8 ppt) at 20 °C with natural photoperiod. Three diets (Control: 10% FO; FO-0: 0% FO; FO-5: 5% FO) were tested in triplicate per salinity. After 83 days, samples of plasma, liver, anterior intestine, gill, and muscle were taken for the same analyses, with gene expression additionally assessed in the intestine and gill. Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test or Welch's ANOVA when variance homogeneity assumptions were not met (Trial 1) and two-way ANOVA followed by Tukey's post hoc test (Trial 2).

## Results and Discussion

In Trial 1, final body weight ($BW_f$) was lower in the FM-5 group (17.15 ± 4.66 g) compared to the Control (18.42 ± 4.71 g) and FM-0 (18.56 ± 4.16 g) groups, with a significant difference between the FM-0 and FM-5 groups ($p < 0.05$; Welch's ANOVA). Feed conversion ratio (FCR) and specific growth rate (SGR) were similar between the Control (FCR: 8.39 ± 1.46; SGR: 0.62 ± 0.08) and FM-0 (FCR: 7.66 ± 1.64; SGR: 0.61 ± 0.12) groups, while FM-5 exhibited a higher FCR (13.11 ± 6.93) and a lower SGR (0.55 ± 0.29). Viscerosomatic index (VSI) and hepatosomatic index (HSI) values showed no significant differences among treatments. No differences were found in plasma glucose and cortisol levels; however, lactate levels were significantly lower in the FM-0 group compared to the Control group ($p < 0.05$; one-way ANOVA). This study indicated that the total replacement of FM with plant protein sources (FM-0 diet) did not significantly affect growth performance, feed efficiency, or somatic indices in juvenile flathead grey mullet compared to the Control diet. These findings align with the natural feeding behavior of mullet, a species well adapted to herbivorous and detritivorous diets (Brusle, 1981; Wassef et al., 2002).

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In contrast, partial replacement (FM-5 diet) led to a significant reduction in $BW_f$ compared to the FM-0 group, suggesting that intermediate FM levels combined with plant-based ingredients may have negatively impacted diet palatability (Gisbert et al., 2016)optimizing weaning diets is of special importance for this species at this particular stage of development. Thus, authors have tested a weaning protocol for wild flathead grey mullet fry (202mg initial body weight. Regarding plasma biochemical parameters, such as lower plasma lactate concentrations were recorded in fish fed the FM-0 diet compared to the control group, potentially reflecting a better metabolic adaptation to the plant-based formulation.

In Trial 2, $BW_f$ did not differ significantly among dietary treatments at either salinity level. Mean values were: Control, 28.96 ± 7.332 g (8 ppt) and 30.79 ± 8.964 g (36 ppt); FO-0, 27.45 ± 10.11 g (8 ppt) and 28.84 ± 11.53 g (36 ppt); FO-5, 28.80 ± 8.847 g (8 ppt) and 28.98 ± 9.920 g (36 ppt). Similarly, no significant differences were observed in FCR or SGR. At 36 ppt, VSI was significantly higher in fish fed the Control diet (9.22 ± 0.91%) compared to those fed FO-0 (7.76 ± 0.55%) and FO-5 (7.55 ± 0.54%) diets ($p < 0.05$). HIS was significantly higher in FO-5 fed fish at 8 ppt (0.80 ± 0.15%) than at 36 ppt (0.64 ± 0.13%) ($p < 0.05$). Plasma glucose levels were significantly higher in FM-5 fed fish at 36 ppt compared to the Control group ($p < 0.05$). A salinity effect was also evident in FO-5 fed fish, with significantly higher glucose concentrations at 36 ppt than at 8 ppt ($p < 0.05$). Plasma lactate levels showed a similar trend. Finally, circulating cortisol was significantly higher in control-fed fish reared at 8 ppt compared to those at 36 ppt ($p < 0.05$). This trial indicated that the total replacement of FO with vegetable lipids did not affect growth performance and feed efficiency in mullet, consistent with their omnivorous feeding habits (Wassef et al., 2002). This is highly positive from a sustainability perspective, supporting the reduction of marine resource dependency. However, metabolic responses varied with diet and salinity, highlighting the need to consider environmental factors in feed formulation.

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**Acknowledgments**

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# INTEGRATIVE OMICS APPROACHES FOR UNDERSTANDING THE FISH LOCAL AND SYSTEMIC IMMUNE RESPONSES TO SINGLE AND CO-INFECTIONS

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## Introduction

Currently, fish production through aquaculture and wild capture is growing as a result of the increased fish protein demand for human consumption. The adverse effects caused by pathogens both in hatchery and grow-out ponds, have decreased aquaculture production and causing high economic losses for the aquaculturalists. Therefore, it is becoming increasingly important to improve fish products in size and value through adequate disease management procedures. A systematic study using omics approaches can help us to understand the complex and sophisticated interactions in the host-pathogen network, leading to identifying new targets and biomarkers for diseases caused by bacteria, viruses, and parasites. The use of different omics approaches is a powerful that can provide new knowledge on host-pathogen interactions and can help us develop novel approaches for disease management and sustainable aquaculture production.

## Materials and methods

We used the two prominent omics approaches proteomics and transcriptomics to study host immune responses and host-parasite interactions in a well-established co-infection disease model. We have investigated how the myxozoans *Myxobolus cerebralis, and Tetracapsuloides bryosalmonae* modulate the host fish immune response after single and co-infections by quantitative proteomics and transcriptomics. For the infection experiment, one group of fish was infected with *M. cerebralis* (Mc group) and another group with *T. bryosalmonae* (Tb group). After 30 day, half of the fish in each group were then co-infected with the other parasite (Mc+ group and Tb+ group). At day 31, the proteomic changes and the transcriptomic modulation in the caudal fins, gills, head cartilage and kidney of rainbow trout were investigated at different time points. The parasite load was determined post exposure in single and co-infection groups.

## Results

In the caudal fins and the gills, multiple transcripts and proteins mostly involved in parasite recognition, tissue repair and the regulation of host immune responses were differentially modulated between the groups in the caudal fin. In the head cartilage and kidney, numerous transcripts and proteins were differentially regulated between the groups. The identified proteins in the head cartilage were primarily involved in host protection against oxidative stress, DNA and RNA synthesis and metabolism. The differentially regulated proteins in the kidney were mainly involved in proteasomal degradation, metabolism and host immunity. Several myxozoans transcripts and proteins involved in parasite development and virulence were identified. The biological processes, molecular pathways and molecular functions of the differentially regulated transcripts and proteins were also analysed. It appears that *M. cerebralis* modulates the rainbow trout immune response in a path that likely synergistically facilitates a subsequent co-infection with *T. bryosalmonae*. Conversely, *T. bryosalmonae* triggers the host immunity apparently by induction of the several B-cell markers including MHC II, B-cell receptor CD22 and Ig-like domain containing protein, which may antagonistically amend the outcome of a subsequent co-infection with *M. cerebralis*.

## Conclusion

The results of the undertaken quantitative proteomic and transcriptomic analysis indicate the successful integration of multiple omics approaches for systemic investigation of biological systems in single and co-infection disease models. The investigations provide novel insights of how myxozoan parasites modulate host immune responses influencing the outcome of co-infections in rainbow trout. The analysis revealed key parasite virulence transcripts and proteins and highlighted potential invasion/evasion strategies during myxozoan infections. The results reveal novel traits of host-parasite interactions and paves the way for the development of novel tools for myxozoan disease management in aquaculture.

# IMMUNOMODULATORY EFFECT OF A DIETARY SUPPLEMENT IN WHITELEG SHRIMP *Litopenaeus vannamei* CHALLENGED WITH *Vibrio parahaemolyticus*

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## Introduction

Whiteleg shrimp (*Litopenaeus vannamei*) have experienced rising production over the past decade, but intensified farming has led to increased susceptibility to diseases such as acute hepatopancreatic necrosis disease (AHPND). This, caused by *Vibrio parahaemolyticus* (VP), severely impacts the hepatopancreas (HP), a key organ for metabolism and immunity. This infection can be detected through the dissection of shrimp HP, where typical signs include a pale appearance and organ atrophy. Due to the economic losses caused by AHPND for shrimp farmers, the use of antibiotics to prevent and treat this infection is common in developing countries. However, the misuse of antibiotics has caused microbial resistance, which poses a significant global threat by reducing the effectiveness of disease treatments. To combat this, alternatives to antibiotics have been explored in shrimp such as essential oils, mannanoligosaccharides and β-glucan, which strengthen shrimp immunity. Additionally, glycerol fatty acid esters have shown antimicrobial properties against Vibrio pathogens. The aim of this study was to evaluate whether a combination of dietary immunomodulators (Marfeed Gut12) can improve the immune resistance of whiteleg shrimp against VP and at which dosage.

Figure 1. Survival rate after challenge

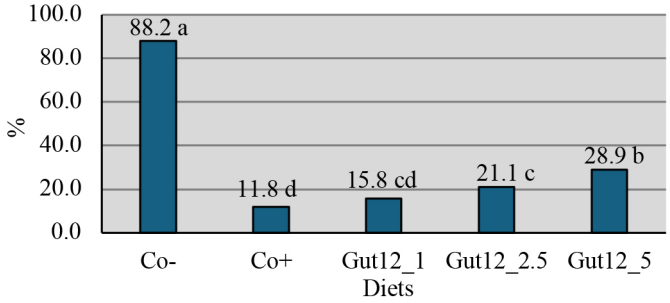
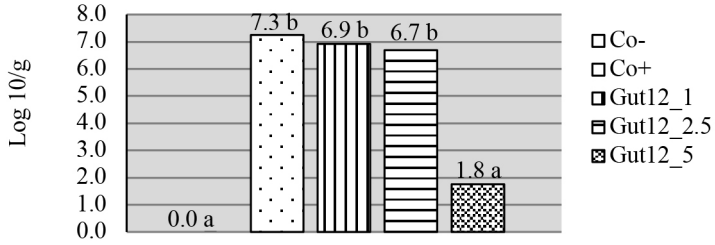



Figure 2. *Vibrio parahaemolyticus* load in hepatopancreas at the end of challenge period




A significant difference between experimental groups is indicated by different letters above the bars ($p < 0.05$).

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Materials and Methods

In a four-replicate setup, five treatments were investigated, including two control groups and three test groups with 30 shrimp (average initial weight of 1.53 ± 0.07 g) per replicate. The controls included an unchallenged negative control (Co-) and a positive control (Co+) challenged with AHPND. The test groups were also challenged and fed a diet supplemented with test product at dosages of 1.0 (Gut12_1), 2.5 (Gut12_2.5), and 5.0 (Gut12_5) g/kg Marfeed Gut12. The 27-day trial comprised a 2-day acclimation period, 14 days of pre-challenge feeding, a 1-day VP challenge, and a 10-day post-challenge observation phase. The challenge was conducted via immersion in water by an $LD_{50\text{-}60/96\text{-}120\ h}$ dose of $VP_{AHPND}$ for all diets, excluding the negative control group. Shrimp survival rates were assessed post-challenge eight times per day. At the end of the challenge, qPCR was used to measure VP quantities in the HP, with samples taken from three shrimp per tank. Data analysis through one-way ANOVA revealed statistically significant differences between mean values at $p < 0.05$.

Results

The findings suggest that dietary supplementation of Marfeed Gut12 significantly enhanced shrimp survival rates in groups challenged with AHPND (Fig. 1). The survival rate, nearly doubled ($p < 0.05$) in the group receiving 5 g/kg of the supplement (28.95 ± 6.8%) compared to the positive control group (11.84 ± 2.63%). Furthermore, the 5 g/kg dosage notably reduced the quantity of VP in the HP compared to other infected groups (Fig. 2).

Conclusions

Incorporating Marfeed Gut12 at 5 g/kg in shrimp diets significantly enhances their resistance and survival against *Vibrio parahaemolyticus*.

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# A NOVEL, ACCESSIBLE SENSOR FOR THE PROMPT DETECTION OF *Vibrio vulnificus* IN AQUATIC ENVIRONMENTS

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## Introduction

Global warming is driving rapid ecological shifts, including changes in the geographical range and virulence of pathogenic bacteria. *Vibrio vulnificus* (Vv), a species linked to warmer waters, has emerged as a key biomarker for climate change, with recent detections in aquatic ecosystems that are traditionally colder. Effective assessment of these changes and the implementation of mitigation strategies require localized environmental surveillance. However, current monitoring methods often rely on complex instrumentation, which limits their widespread applicability. Consequently, there is a pressing need for novel detection strategies that are portable, affordable, and effective.

## Materials and Methods

This study describes the monitoring of a Mediterranean coastal wetland using a rapid and straightforward technology for detecting Vv in water samples (Figure 1). The method integrates an enrichment step with isothermal amplification of the *vvhA* gene via recombinase polymerase amplification (RPA). The final detection is performed visually on a lateral flow strip, which is incorporated into a microfluidic chip.

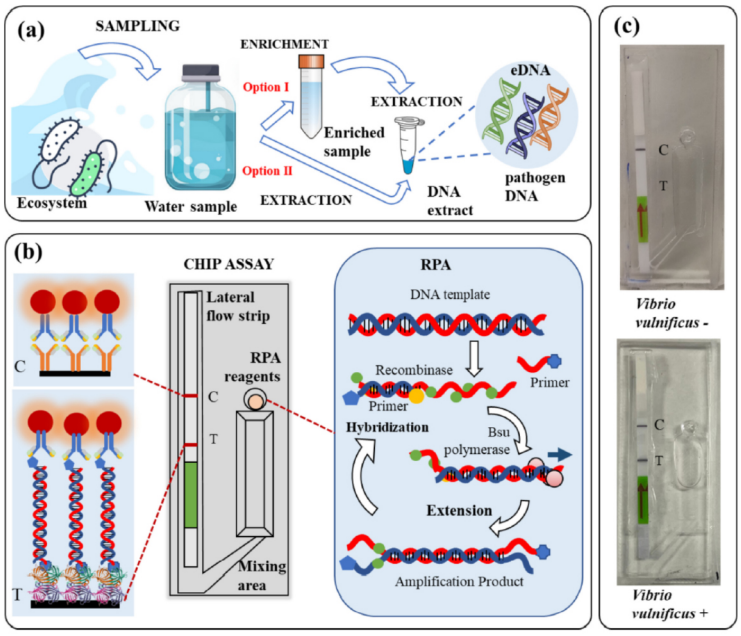


***Figure 1. Overview of Vv detection in environmental samples****. (a) Schematic workflow for samples collected from aquatic ecosystems. An enrichment step (Option I) is required for low Vibrio concentrations, while high concentrations can bypass this step (Option II). (b) Assay principle for on-chip amplification and detection, which uses recombinase polymerase amplification (RPA). (c) Chip images showing the visual detection of the target bacterium in lake water samples, where a T (test) line indicates the presence of Vv and a C (control) line confirms a successful assay. Figure taken from Quintero-Campos et al. (2025); DOI: 10.1016/j.scitotenv.2025.179791.*

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**Results**

The sampling campaign identified areas within the wetland where Vv concentrations surged by up to 300 times, notably in brackish zones and at points of water inflow or outflow. The new system's results were a perfect match with those from traditional PCR and electrophoresis, confirming its reliability. This innovative tool has the potential to pinpoint microenvironments that support or inhibit bacterial growth and could be a vital component of global surveillance strategies for climate change-related diseases.

**Conclusion**

The implemented sensor demonstrated high sensitivity, effectively identifying Vv blooms in environmental waters that could pose a risk to public health.

**Acknowledgements**

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# MULTIGENERATIONAL TEMPERATURE EFFECTS ON PATERNAL-TO-OFFSPRING EPIGENETIC INHERITANCE IN SEA BASS

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## Introduction

Environmental changes can trigger epigenetic modifications that influence gene expression and, consequently, the phenotype. However, key questions remain about the mechanisms underlying epigenetic inheritance and the extent to which environmentally induced epigenetic marks are transmitted across generations. In particular, the potential role of epigenetic mechanisms —especially DNA methylation—in facilitating short-term adaptation is still under investigation. To address these questions, we examined how temperature influences genome-wide DNA methylation patterns, with a specific focus on the sex phenotype and genes involved in sex determination and differentiation, and assessed the potential transmission of these modifications to the offspring.

## Materials and Methods

We used the European sea bass (*Dicentrarchus labrax*), a fish species with a polygenic and temperature-sensitive sex determination system, to investigate the effects of temperature on DNA methylation. Genome-wide profiles were obtained at single-nucleotide resolution using reduced representation bisulfite sequencing in 66 individuals from five families across two generations ($F_0$ and $F_1$). Individuals were exposed to control (16 °C, C) or elevated (21 °C, T) temperatures between 12 and 60 days post-fertilization in a multigenerational experimental design. Four temperature exposure regimes were applied to the $F_1$ generation: control (CC), ancestral exposure via sires (TC), developmental exposure in offspring (CT), and dual exposure (TT).

To assess the effects of temperature and sex on DNA methylation, we performed the following analyses: First, for each region covered, we applied a generalized linear model with a binomial distribution to methylation count data, using $F_0$ and $F_1$temperature treatments as fixed effects and correction for multiple testing. Analyses were performed separately for testes and ovaries. This approach allowed us to classify regions into five categories: no effect, $F_0$-only effect, $F_1$-only effect, additive effect (same direction of change in $F_0$ and $F_1$ without interaction), and interactive effect (significant interaction between $F_0$ and $F_1$, regardless of direction of change). These regions were further filtered based on differential methylation, using sex and sire as covariates. Second, we investigated temperature effects, specifically focusing on a panel of sex-related genes. And, third, we assessed the proportion of stable (constitutive) versus environmentally-induced epigenetic marks between generations to evaluate the potential for inheritance of methylation changes.

## Results and Discussion

In terms of phenotypic sex, we observed a significant increase in the proportion of males in temperature-treated groups, as expected. However, when comparing the dual-exposure group (TT) with the control group (CC), differences in sex ratios approached but did not reach statistical significance ($P = 0.051$). Regarding other traits, males exhibited significantly higher body weight and length in response to temperature treatment when compared to CC males, with the TT group showing the highest values. In contrast, temperature-resistant females also displayed an increase in body size under temperature treatment, but this effect was not significant when comparing CC and TT groups.

Analysis of DNA methylation data revealed marked sex-specific differences in the distribution of regions across model-defined categories. Notably, only 5.1% of regions in males and 3.7% in females exhibited an additive temperature effect. In contrast, a substantial proportion of regions —37.0% in males and 31.1% in females— showed qualitative interactions, where the direction of the temperature effect differed between generations ($F_0$ and $F_1$). This pattern suggests the presence of compensatory epigenetic mechanisms in response to repeated temperature exposure.

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We are currently working on analyses on neomales (genetic females that developed as phenotypic males); results from these individuals will provide further insight into temperature- and sex-specific methylation dynamics.

Additionally, we identified several key sex-related genes whose methylation patterns were significantly affected by temperature in a regime-dependent manner. Furthermore, we found that while a substantial portion of epigenetic marks were stably transmitted from sires to offspring, a small but noteworthy fraction (~5%) of inherited marks were temperature-induced and transmitted to the following generation. These findings offer new insights into the extent of environmentally-driven epigenetic inheritance.

**Conclusions**

Overall, our study underscores the influence of temperature on the epigenetic landscape, with important implications for understanding plasticity, adaptation, and epigenetic effects transmitted across generations in the context of global climate change.

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# ATLANTIC SALMON ASSOCIATED RESISTOMES: A COMPARISON BETWEEN WILD AND CAPTIVE POPULATIONS

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**Introduction**
Microbial communities inhabiting fish-associated niches and surrounding water play a crucial role in host health, environmental resilience, and pathogens persistence. However, little is known about how these communities mediate the coexistence of antibiotic resistance genes (ARGs) and metal resistance genes (MRGs), particularly across wild and captive environments. As part of the *BlueRemediomics* project, we investigated the microbiota resistome of Atlantic salmon (*Salmo salar*) from freshwater and marine environments by sampling the skin, gut, feces and surrounding water. Fish were collected from sea cage farms (captive) and from rivers or fjords (wild), all corresponding to the smolt stage and of comparable size.

**Materials and methods**
DNA was extracted using the Zymo Research Miniprep kit following the manufacturer's recommendations. Total genomic DNA from 40 samples was then processed for metagenomic sequencing on the NovaSeq 6000 PE150 platform, targeting ~10 million reads per library. Raw reads were quality-checked using *fastp*, and host DNA contamination was removed with *Bowtie2* using the reference genome GCF_905237065.1. ARGs and MRGs were annotated using the PanRes database (v1.0.1) and the KMA tool. The same DNA samples were used for metabarcoding sequencing targeting the V3–V4 regions (341F/R805) of the 16S rRNA gene, using the Illumina NextSeq PE250 platform. Genetic and taxonomic diversity was analyzed using ecological indices (alpha and beta diversity) and compared across sample types, environments, and rearing systems.

**Results**
Results showed that the abundance and diversity of ARG classes was significantly influenced by sampling origin and type. Mucosal niches (skin and gut) harbored the highest diversity of ARG–MRG combinations in both captive and wild fish. Water samples exhibited distinct resistance profiles, while fecal samples contained only a marginal presence of resistance genes. This highlights the importance of sample type selection when profiling ARG–MRG communities, with more tightly fish-associated microbiota carrying more diverse resistance families.

Among clinically relevant families, aminoglycoside, fluoroquinolone, and beta-lactamase resistance genes were the most dominant. Notably, aminoglycoside profiles mirrored the abundance of copper resistance genes, particularly in skin microbiota. Furthermore, genera such as *Stenotrophomonas* and the NS5 marine group were positively associated with several specific resistance genes, suggesting potential co-selection. Future work should include the isolation and comprehensive characterization of these Atlantic salmon–associated strains to better understand their clinical relevance in both captive and wild populations.

**Conclusion**
The present findings highlight the influence of the ecosystem niche on the resistome development, including the environmental pathways that may facilitate ARG–MRG co-selection. Our results aim to contribute towards the improvement of targeted monitoring and mitigation strategies to reduce the dissemination of resistance genes while supporting sustainable aquaculture practices.

**Acknowledgments**
This project has been funded by BlueRemediomics-Grant No.101082304, Horizon Europe Programme 2022. Fish were sampled from Lerøy´s land and sea facilities in Norway.

# DIETARY SUPPLEMENTATION WITH A NATURAL ANTIOXIDANT MODULATES MUSCLE STRUCTURE AND FRESHNESS PHYSICAL PARAMETERS UNDER CHRONIC STRESS IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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## Introduction

One of the objectives of the project PHYTOWELFISH aims to determine if a phytogenic antioxidant, AquaNeox (TECNOVIT-FARMFaes Ltd.), can improve the physiological status in European seabass (*Dicentrarchus labrax*) raised at high stocking density. Phytogenics, which are plant-derived compounds like carotenoids or phenolic compounds, are used in aquaculture to protect tissues and improve animal welfare (Hu et al., 2025). This study will focus on how AquaNeox and stocking density affect skeletal muscle development, specifically the formation of new muscle fibers (hyperplasia) and the growth of existing ones (hypertrophy) (Alami-Durante et al., 2010; Zho et al., 2018). While various factors influence muscle growth, the combined effects of diet and stocking conditions in muscle physical parameters and cellularity are not yet well understood.

## Materials and methods

European seabass (*D. labrax*) fish (n = 480; $w_i$ = 105 ± 30 g) were divided into four groups, each with three replicates. The groups were based on two factors: diet (control or AquaNeox at 0.05%) and stocking density (low-LD: 5 kg/m$^3$ or high-HD: 37 kg/m$^3$). After two months, muscle samples from 12 fish per experimental group were taken to analyze the combined effects of stocking density and dietary supplementation on physical parameters such pH, torrymeter, color, and moisture. Additionally, muscle samples from 9 fish per group were taken below the dorsal fin, sectioned and fixed in 10%-formalin. These sections were stained with hematoxylin/eosin to photograph whole muscle cross-sections in a light microscope (Nikon Eclipse Ci-L) with a Jenoptik ProgRes CT5 camera. Then, histomorphometric parameters such muscle fiber perimeter, area, and Feret's diameter were measured using FIJI software in IMAGEJ (50 measurements per fish). Fiber diameters were then grouped into five classes to determine hyperplasia, hypertrophy rates, and fiber density.

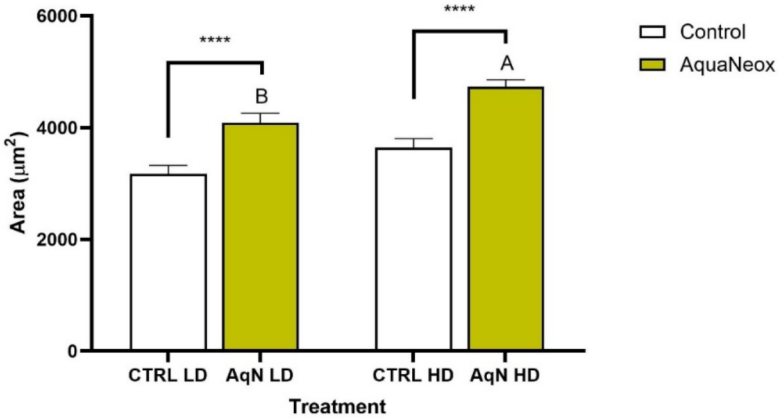


**Figure 1.** White muscle transverse area (µm$^2$) in European seabass (*Dicentrarchus labrax*) ($n$ = 9 animals per group). Different capital letters represent significant differences between stocking densities within the AquaNeox diet ($p < 0.05$). Asterisks indicate significant differences between diets within the same stocking density ($p < 0.0001$).

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## Results and discussion

The study focused on key physical parameters of muscle quality and cellularity. At the end of the study, no significant differences were found across any experimental group for muscle moisture (65 ± 4%), pH (7.3 ± 0.1), or Torrymeter values (13.5 ± 0.4). Low-density groups (CTRL-LD and AquaNeox-LD) tended to have higher L* (lightness) values, regardless of AquaNeox, suggesting lighter muscle compared to high-density groups (CTRL-HD and AquaNeox-HD). There were no significant differences in a* (green/blue shift) or b* (redness) values across any of the groups, regardless of the AquaNeox treatment or density. Regarding muscular cellularity, the study found no evidence of hyperplasia (new muscle fiber formation), based on fiber density and distribution. Instead, a clear trend toward hypertrophic growth was observed, characterized by an increase in fibers with diameters exceeding 45 $\mu$m and reduced recruitment of new fibers. While stocking density did not significantly affect muscle cellularity, AquaNeox treatment significantly increased muscle fiber area (Figure 1), perimeter, and Feret's diameter across both low and high stocking densities. This strong hypertrophic effect of AquaNeox aligns with previous studies with antioxidants, in which also increased fiber diameter and related gene expression (Thomazini Zanella et al., 2021).

## Conclusions

Adding AquaNeox to the diet of European seabass effectively promoted muscle growth through hypertrophy (increase in fiber size) by increasing fiber area, perimeter, and diameter. It did not, however, lead to hyperplasia (formation of new muscle fibers). This effect of AquaNeox is independent of stocking density, which had no significant impact on muscle cellularity or physical parameters such as moisture, pH, or color. Therefore, AquaNeox is a promising dietary supplement for improving muscle quality in European seabass.

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# EFFECT A NATURAL NUTRACEUTIC ANTIOXIDANT ON INCREASING THE SHELF LIFE OF FISH FILLETS FROM EUROPEAN SEABASS (*Dicentrarchus labrax*)

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## Introduction

Global demand for high-quality aquatic products necessitates effective freshness and shelf-life strategies (Yeşilsu et al., 2025). Consumers favor natural, plant-derived phytochemicals over synthetic additives due to the latter's antioxidant and antimicrobial properties, which can prevent degradation and inhibit microbial growth, thereby improving food quality (Biswas et al., 2023). For example, AquaNeox (TECNOVIT-FARMFaes Ltd.), a phytogenic nutraceutical, provides substantial antioxidant benefits and can be incorporated into the diet of fish to reduce oxidation. In the PHYTOWELFISH project it was evaluated the potential of this additive to increase the quality of European seabass (*Dicentrarchus labrax*) meat, based on the hypothesis that dietary natural antioxidants would counteract oxidative spoilage and improve the freshness and shelf life of processed fish fillets.

## Materials and Methods

European seabass adults (*D. labrax*) (initial mean weight 300 g) were divided into three experimental groups, in duplicate, according to their diet: i) control group, ii) diet supplemented with AquaNeox at low concentration (0.05%), iii) and supplemented with AquaNeox at high concentration (0.1%). After 64 days, fish were euthanized using ice slurry, to mimic commercial practices, and kept on ice for 24-48 hours to simulate commercial conditions, then eviscerated, processed into fillets (with bone), packaged, and stored under refrigeration at 4 °C. Freshness and shelf-life were monitored through periodic sampling at day 0, 5, 8, 12, 15, and 20. Analyzed parameters included pH, water activity, color, texture, TVB-N and TMA as physicochemical indicators of freshness; LPO and ORAC as indicators of oxidative stability and the effectiveness of antioxidant strategies; and microbiological analysis as detection of pathogens/indicator microorganisms of spoilage.

## Results

The results showed that pH values generally increased over storage time across all groups, with AquaNeox having a slightly favourable effect. It helped maintain water activity and preserve the product's initial colour better, specifically reducing yellowness (b). Adding 0.1% AquaNeox resulted in the lowest total colour difference (ΔE) over time (Figure 1a). AquaNeox also mitigated the increase in spoilage indicators such as total volatile basic nitrogen (TVB-N) and trimethylamine (TMA), with the 0.1% concentration performing best (Figure 1b). The additive demonstrated good antioxidant capacity, resulting in low lipid peroxidation (LPO) and improved preservation, particularly with 0.1% AquaNeox on days 8 and 15 (Figure 1c–d). Although microbiological quality remained stable below spoilage levels for the first eight days, mesophilic bacteria increased at a similar rate across all groups from day 15 onwards, though all samples met international standards (Regulation EC No. 2073/2005). Texture parameters remained stable and were not significantly affected by the treatment.

## Conclusions

This study demonstrates that supplementing with AquaNeox, particularly at concentration of 0.1%, could extend the shelf life of sea bass fillets by reducing spoilage markers (TVB-N and TMA), oxidative damage (ORAC) and color loss. This supports the use of AquaNeox as a nutraceutical in aquaculture.

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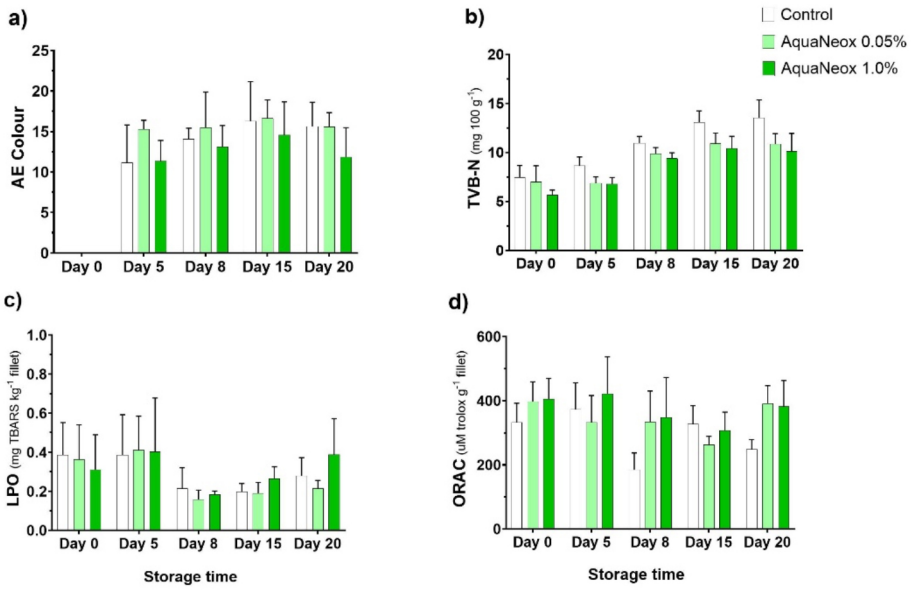


**Figure 1.** Freshness and oxidative stability parameters determined in European seabass (*D. labrax*) fillets from fish fed for two months with control diet or supplemented with AquaNeox at 0.05% and 0.1% concentrations ($n$ = 6). (**a**) Color variation (ΔE). (**b**) Total Volatile Basic Nitrogen (TVB-N), (**c**) Lipid Peroxidation (LPO), (**d**) Oxygen Radical Absorbance Capacity (ORAC).

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# FIRST REPRODUCTIVE EVENT OF HATCHERY-PRODUCED *Ostrea edulis* FROM THE MAR MENOR LAGOON

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## Introduction

The flat oyster *Ostrea edulis* is a native European species known for its significant role in ecosystem services such as habitat creation and biofiltration. Historically abundant across the northeastern Atlantic and the Mediterranean basin, its populations have drastically declined due to overexploitation, habitat loss, and environmental degradation (Thurstan et al., 2024).

*O. edulis* is increasingly being used in restorative aquaculture, which, when properly implemented, offers a promising and sustainable approach to ecosystem restoration. In the Mediterranean Sea, specifically in the southeast of Spain, the Mar Menor Oyster Initiative (MMOI, https://noraeurope.eu/spain-the-mar-menor-oyster-initiative/) is being carrying out. This initiative aims to study the nutrient extraction capacity of *O. edulis* in the hypersaline Mar Menor lagoon and to develop tools necessary for future restoration and bioextraction actions (Albentosa et al., 2023).

The main objective of this study is to increase the *O. edulis* stock to support ecosystem restoration efforts in the Mar Menor and to validate the reproduction of hatchery-produced oysters in 2022 (Hernandis et al., 2023), confirming their viability for successful breeding.

## Material and methods

The reproduction of the oysters was carried out from March to May 2024 according to its natural reproductive season in the Mar Menor lagoon (SE Spain). It took place at the IEO-CSIC experimental hatchery using 69 broodstock individuals: 29 wild oysters from the lagoon (WB) and 40 hatchery-produced in 2022 (HB) in the same experimental hatchery.

## Results and discussion

Spawning followed natural thermal cycles, producing a total of 36 spawns and 59.8 million larvae, 48% of which originated from HB (Figure 1). These findings confirm for the first time that individuals bred in hatchery in 2022 (Hernandis et al., 2023) are reproductively viable. Larvae from WB were significantly larger (mean = 168.5 $\mu$m) than those from HB (mean = 153.3 $\mu$m), and their survival to settlement was also higher (54% vs. 21%, respectively). However, the survival rate to postlarvae stage was higher (24%) for those larvae from HB than those from WB (16%). The observed differences in fecundity and larval performance are consistent with age-related reproductive maturity: WB individuals were older and fully mature

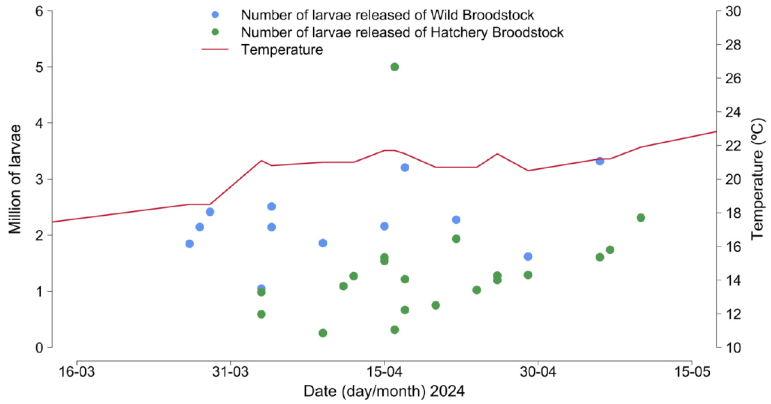


Figure 1. Monthly spawns per broodstock origin related with the temperature.

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possessing greater energy reserves and more developed gonads, whereas HB were in their first reproductive cycle after reaching sexual maturity. This may influence reproductive outcomes, with older individuals typically having larger energy reserves and more developed reproductive organs (Bayne, 2017).

This study demonstrates the reproductive potential of both wild and hatchery *O. edulis* from the Mar Menor, highlighting the viability of establishing a reliable stock of locally adapted native oysters. This integrates aquaculture into restoration frameworks allowing large-scale actions in the lagoon, aligned with global efforts to recover native oyster habitats and the ecosystem services they provide.

**Acknowledgments**
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# TRANSCRIPTOMIC PROFILING OF SPERMATOGENESIS IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*) ACROSS THE REPRODUCTIVE CYCLE

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**Introduction**

The European sea bass (*Dicentrarchus labrax* L.) is a key aquaculture species in Mediterranean countries. In farming conditions, males often undergo precocious puberty, which negatively affects their growth (Felip et al., 2006). Understanding the molecular mechanisms that regulate testicular maturation is essential for improving reproductive management and minimising growth retardation. This study aims to characterise the transcriptomic landscape of spermatogenesis across the annual reproductive cycle in this economically important fish species.

**Materials and methods**

Testes were collected during the second year of life covering the six stages of spermatogenesis that comprise the entire annual reproductive cycle (Begtashi et al., 2004): Stage I (immature), Stage II-SIV (early, mid and late recrudescence), Stage V (fully spermiating), and Stage VI (post-spawning). Samples were processed for histological analysis and directional RNA sequencing (RNA-seq). The histological study enabled precise staging and quantification of germ cell types in the testes. Moreover, it allowed for the selection of eighteen samples (three per stage) that were further used for transcriptomic profiling by RNA-seq (Love et al., 2014).

**Results**

Transcriptomic analysis enabled the *de novo* assembly of the sea bass testis transcriptome and the identification of differentially expressed genes (DEGs) across all stages compared to the basal stage (SI). Early spermatogenesis (SII vs SI) was characterised by the activation of gonadotropin and steroid hormone signalling pathways, promoting the proliferation of spermatogonia A. In mid-spermatogenesis (SIII vs SI), genes involved in mitosis, DNA replication, and chromatin remodelling were upregulated, reflecting the intense proliferation of spermatogonia B and spermatocytes I. Spermiogenesis (SIV vs SI) was characterised by a DEG enrichment in pathways associated with flagellar assembly, chromatin packaging, and transcriptional regulation. During spermiation (SV vs SI), DEGs included genes involved in sperm maturation and release, such as those related to calcium signalling and proteolytic activity. Finally, testicular regression (SVI vs SI) was characterised by the upregulation of genes involved in vascular remodelling and immune response.

**Discussion**

Bulk RNA sequencing data revealed the presence of DEGs and functional pathways associated with steroidogenic activity, and mitotic and meiotic proliferation of spermatogonial stem cells at the onset of spermatogenesis (SI-SIII). This pattern is consistent with Gene Ontology enrichment analyses, which are strongly associated with DNA replication and recombination processes. Notably, this stage also featured a downregulation of *amh* and *cyp26a*, highlighting their importance in initiating and driving the progression of spermatogenesis (Skaar et al., 2011; Blázquez et al., 2017). During late spermatogenesis (SIV-SV), the expression of sperm-specific DEGs was increased, suggesting a mechanism for sperm-chromatin packaging that relies on a diverse group of histones (Barrachina et al., 2018). In line with previous studies, the regression stage (SVI) that marks the end of the spermatogenic cycle was characterised by testicular remodelling, accompanied by increased expression of immune-related genes and enhanced vasculogenesis (Valero et al., 2015). Furthermore, developmental function analysis led to the identification of other novel spermatogenesis-related genes across the different stages.

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**Conclusions**

This study provides the first comprehensive transcriptomic atlas of spermatogenesis in the European sea bass, identifying key molecular markers and regulatory pathways across the annual reproductive cycle. These findings shed light on the genetic and molecular mechanisms underlying spermatogenesis, an essential process for ensuring reproductive efficiency and optimising growth in cultured fish species.

**Acknowledgements**

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# ORAL DELIVERY IN MOLLUSKS: PRELIMINARY RESULTS

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**Introduction**

Bivalve mollusk aquaculture is very appealing due to its benefits for human nutrition, the economy, and the environment. However, production is currently constrained by issues like feed deficiencies, diseases, and quality concerns (FAO, 2024). Recent advancements in microencapsulation technology offer promising opportunities to enhance bivalve farming. Microparticles (MPs) can effectively deliver important molecules in aquaculture, such as peptides or antibiotics, by protecting these substances from degradation in acidic environments (Shah, et al., 2020).

In this study, MPs serve as delivery vehicles for both small molecules and nucleic acids. Main component of MPs were small unit vesicles or SUV liposomes (Walde et al., 2001) combined with graphene oxide and/or polyethylenimine polyplexes, each component played different structural and functional roles. Graphene oxide (GO), a biocompatible two-dimensional nanomaterial, acted as a flat assembly platform, aiding the integration of liposomes and other elements. Branched polyethylenimine 25KDa (PEI), a cationic polymer recognized for its gene delivery properties, was used to compact a plasmid encoding green fluorescent protein (GFP) forming polyplexes (Deng et al., 2009; Kim et al., 2011; Sercombe et al., 2015). The main objective of this project was to assess the ability of three different MPs to serve as delivery vehicles for small molecules and nucleic acids to bivalve mollusks.

**Materials and Methods**

Different formulations of MPs were prepared using SUV liposomes, GO and polyplexes (PP) composed of PEI and a plasmid DNA encoding GFP as the nucleic acid cargo. Three types of MPs were tested: (1) GO/liposomes, (2) PP/liposomes, and (3) PP/GO/liposomes. For experimental validation, all liposomes were loaded with carboxyfluorescein (CF), except the control group, whose liposomes were not charged with CF. The size distribution of each kind of microparticles was analyzed using a Corning Videodrop (Corning Incorporated, USA). The delivery properties of CF or the GFP plasmid were tested *in-vivo* in the Mediterranean scallop (*Pecten jacobaeus*).

The *in-vivo* experiment included three treatment groups and one control group, with a total of twelve animals—three individuals per treatment and three in the control group. Each animal was kept individually in a 1L tank, where the different microparticle formulations were added. Fecal samples were collected over a 48 to 72-hour period. After this, the animals were sacrificed, and tissue samples were collected and preserved in 1 mL tubes containing RNAlater, then stored at 4 °C until further analysis. The collected tissues included gills, mantle, crystalline style, adductor muscle, digestive gland, and gonad.

All tissue samples were examined using an InCellis fluorescent microscope (Bertin Technologies, Montigny-le-Bretonneux, France). Total RNA was extracted from all tissues using the RNeasy Kit (QIAGEN, Hilden, Germany) following the manufacturer's standard protocol. RNA concentration was determined with a BioPhotometer D30 (Eppendorf, Hamburg, Germany). To eliminate genomic DNA contamination, total RNA was treated with DNase I (New England Biolabs, MA, USA). Complementary DNA (cDNA) was synthesized from total RNA using the LunaScript RT SuperMix Kit (New England Biolabs). Quantitative PCR (qPCR) was performed on a CFX96 Touch Real-Time PCR System (Bio-Rad, CA, USA) using *Pecten jacobaeus* 18S rRNA as the reference gene (forward: TCGATGGTACGTGCTATGCC; reverse: CCTTGGATGTGGTAGCCGTT) and EGFP as the target gene (forward: GACGTAAACGGCCACAAGTT; reverse: AAGTCGTGCTGCTTCATGTG).

**Results and Discussion**

Three different formulations of MPs were successfully synthesized. The median diameters were 153 nm for GO/liposomes, 163 nm for PP/liposomes, and 180 nm for PP/GO/liposomes. The major size means of the PP/GO/liposomes complexes likely reflects the increasing supramolecular structure complexity.

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Fluorescence was detected in all samples when observed under a fluorescent microscope, except for the crystalline style samples from the control group and one crystalline style sample from the LPG treatment. This may be because the crystalline style mainly consists of extracellular enzymes with few active cells, limiting the expression of fluorescence. Nonetheless, the detected fluorescence confirms successful ingestion of the MPs and release of their encapsulated content beyond the digestive tract, with visible signals in the gills, mantle, adductor muscle, digestive gland, and gonad, suggesting possible diffusion of the released compounds. Since the MPs used in the control group lack CF, the fluorescence observed is probably due to the internalization of the MP into cells and the expression of the green fluorescent protein GFP (Deng et al., 2009; Kim et al., 2011).

**Conclusion**

In conclusion, the study successfully engineered and synthesized different microparticle formulations with varying sizes in the nanometer range and different structural complexity. Fluorescence microscopy confirmed the effective ingestion and distribution of these microparticles and their contents across multiple bivalve tissues. Notably, the fluorescence observed in the control group, which lacked carboxyfluorescein, constitutes evidence of cellular internalization of the microparticles and expression of the GFP encoded by the plasmid. Preliminary qPCR results support this finding, showing positive amplification in two samples. Further qPCR analysis will offer a clearer understanding of the gene delivery and expression efficiency of the different microparticle formulations.

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# COMPARATIVE ANALYSIS OF GUT MICROBIOTA RESPONSES TO DIETARY INSECT OIL IN EUROPEAN CATFISH VERSUS HYBRID AFRICAN CATFISH

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**Introduction**
Studies testing the applicability of insect oils as alternatives to fish oil in aquaculture have demonstrated positive effects related to lipid metabolism and fish immunity. However, the impact of insect oil on the gut microbiota of fish remains largely unexplored. In this study, we examined the gut microbiota of European catfish (*Silurus glanis*) and hybrid African catfish (*Clarias gariepinus* × *Heterobranchus longifilis*) fed extruded experimental diets containing black soldier fly larvae oil, comparing them to fish fed a traditional fish oil-plant oil-based diet. The aim was to assess how dietary insect oil influences the composition of gastrointestinal microbiota.

**Materials and methods**
Fish were fed with three isonitrogenous (430 g/kg) and isolipidic (110 g/kg) diets (a control and two insect oil (IO) based diets). The main differences between the diets were the oil source and physical properties of the pellets (sinking feed for European catfish, floating feed for African catfish). The control (CTR) diet contained a mixture of fish oil and rapeseed oil (1:1 ratio), IO50 diet contained 50% black soldier fly larvae oil (BSFLO) replacing fish oil and rapeseed oil mixture, and IO100 diet contained only BSFLO (100%). The first feeding trial (T1) involved a total of 630 European catfish juveniles (average body weight of 28.1 ± 0.17 g), randomly distributed into three dietary groups (CTR, IO50, and IO100), each replicated three times (70 fish per tank), and reared in a recirculation aquaculture system at 24.5 °C for eight weeks. A second trial (T2) involved 900 juvenile hybrid African catfish (average weight: 29.1 ± 1.69 g), similarly treated as the first trial. At the end of the feeding period, intestinal fecal samples were aseptically obtained from individual fish specimens and subjected to DNA extraction, amplification of the V3-V4 region of 16S rRNA, sequencing using an Illumina MiSeq sequencer, and analysis of raw sequence reads. The raw 16S rRNA gene sequence files and metadata were deposited at the NCBI SRA database under the BioProject PRJNA1222409.

**Results**
The alpha diversity (Shannon, Simpson, Pielou and Chao1) of intestinal microbiota in groups of European catfish fed the CTR, IO50, and IO100 were similar. Similar results were also obtained between dietary groups (CTR, IO50, and IO100) of hybrid African catfish. However, the Shannon and Pielou indices were significantly higher in hybrid African catfish than observed in European catfish. Regardless of the diet, the intestinal bacterial community of European catfish was dominated by either Fusobacteriota or Spirochaetota which together accounted for over 99% of the total bacterial abundance while that of hybrid African catfish was dominated by Fusobacteriota in all samples (>75% of total abundance). Other bacterial phyla including Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota, and Verrucomicrobiota, occurred with relative abundances <1% in European catfish and <1% to 24.2% in hybrid African catfish. The abundances of these phyla were not significantly influenced by the inclusion of insect oil in the fish diets. The Firmicutes to Bacteroidota ratio, known to be associated with enhanced oxidative response and more resistance to pathogens was higher in groups fed insect oil-based diets (IO50 and IO100) than those fed the control diet (CTR), with significant results obtained for hybrid African catfish. Fusobacteriota was dominated by the genus *Cetobacterium* in both fish species. The genus *Brevinema* was the most dominant in the phylum Spirochaetota and was only identified in faecal samples of European catfish in varying abundances. The numerically dominant Firmicutes in European catfish was *Exiguobacterium* while *Romboutsia* was the dominant Firmicute in hybrid African catfish. There were no major differences in the abundance of these genera between fish fed the CTR, IO50, and IO100.

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The highest number of amplicon sequence variants (ASVs) was shared between the dietary groups for each fish species and between the fish species fed a particular diet. Replacement of the CTR diet with insect oil-based diets led to an increase in the number of unique ASVs from 28.2% to 38.3% in the IO50 and 35.4% in IO100 groups of European catfish and a decrease from 29.8% in the CTR to 17.2% in IO50 and 22.8 in IO100 groups of hybrid African catfish. Most of the ASVs shared between the two species before and after replacement of the CTR with insect oil-based diets, could be core members of these species. These were mainly affiliated to the genus *Cetobacterium* (over 87% of the shared ASVs), the rest being affiliated to *Corynebacterium*, *Cutibacterium*, *Escherichia-shigella*, *Exiguobacterium*, *Bifidobacterium*, *Staphylococcus*, and other members of the family Comamonadaceae. ASVs unique to hybrid African catfish before and after replacement of the CTR diet with insect oil-based diets were mainly affiliated to the genera *Romboutsia, Plesiomonas, Turicibacter, Clostridium sensu stricto 1, Aurantimicrobium, Terrisporobacter,* and *Epulopiscium*. In European catfish, most of the unique ASVs identified before and after replacement of the CTR diet belonged to the genera *Brevinema,* and *Leifsonia*. The differences in bacterial functional composition between the dietary fish groups were similar for each species. The relatively abundant bacterial functions were associated with metabolism, environmental information processing, cellular processes, and genetic information processing.

**Conclusion**

Overall, these results indicated that complete substitution of fish oil or vegetable oils with black soldier fly larvae oil (BSFLO) is possible in the practical diets of siluriform fish, without causing deleterious effects on gastrointestinal microbiota. The observed trends in the Firmicutes/Bacteroidota ratio across the dietary groups, indicated that BSFLO exhibits antioxidant and anti-inflammatory effects closely associated with the increased Firmicutes/Bacteroidota ratio. Although it is unlikely that the *Brevinema* recorded in this study could be an opportunistic pathogen in European catfish, further studies are required to fully understand the effects of such variations in *Brevinema* abundance on the health of European catfish in aquaculture.

**Acknowledgement**

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# GROWTH PERFORMANCE OF JUVENILE TILAPIA *(Oreochromis niloticus)* AT DIFFERENT DENSITIES USING BLUEBERRY *(Vaccinium corymbosum L., 1753)* UNDER EXPERIMENTAL DIET CONDITIONS

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The Tilapia *(Oreochromis niloticus)* aquaculture production is the second most produced aquatic species worldwide and is also extremely important in the production of animal protein. There is interest in using plant-based products in diets, to increase productive performance, fish health, to decrease stress in culture and to support food safety. The importance of blueberry flour (BF) lies in its antioxidant and nutraceutical properties. The objective of this research is to evaluate the effect of including blueberry flour as a feed additive, in tilapia's O. niloticus health farmed in different densities at an experimental level.

A bioassay was performed for 3 weeks in tubs with 32, 40 and 56 organisms per treatment, commercial feed plus 3% BF additive was supplied, they were stocked with an approximate weight of 2.0 grams, with a control density of 25 organisms where BF was not supplied, only commercial feed. Physicochemical parameters, water quality, and hematological analysis were monitored.

The biochemical analysis results show there were differences.

The physicochemical parameters were within optimal range, the temperature was held at 28.0°C ± 2.0, dissolved oxygen at 6 ± 0.8 mg/L and pH at 8.3 UPpH. There were no significant differences ($P<0.05$) between treatments, with the greater organism density consisting of 32 and 24 organisms per square meter.

# ASSESSING THE POTENTIAL BENEFITS OF INTEGRATED MULTI-TROPHIC AQUACULTURE OF *Ulva* spp. AND *Mytilus edulis*

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## Introduction

Bivalves and seaweed extract organic and inorganic compounds from their surrounding environment, including carbon in its particulate or dissolved form, through biocalcification and photosynthesis processes, respectively. Seaweed also absorbs dissolved nutrients such as nitrogen and phosphate (Hurd et al., 2022, Jia *et al*., 2023). In the UK, both seaweed and bivalve monocultures are practiced, with bivalve aquaculture being more widespread and developed. However, instances of co-location of bivalves and seaweed aquaculture in an Integrated Multi-Trophic Aquaculture (IMTA) setting are limited. Initial studies on the co-cultivation of bivalves and seaweed have shown that CO2 and nutrients produced by bivalves (from pseudo-faeces) enhance seaweed growth, while seaweed's CO2 uptake reduces water acidification, thereby improving the growth rates of bivalves' shells and tissues (Young *et al*., 2022). This copilot study aimed to provide further evidence of the benefits of co-locating green seaweed (*Ulva* spp.) with blue mussels (*Mytilus edulis*) in a Recirculating Aquaculture System (RAS), focusing on both species' growth and water quality parameters.

## Materials and Methods

A total of 9 medium tanks (350L) each associated with its own RAS system were set up. Three distinct conditions were established in triplicate: the cultivation of mussels alone, the co-cultivation of mussels and *Ulva* spp., and the cultivation of *Ulva* spp. alone. Tanks with mussels housed a total of 20 individuals each and tanks with *Ulva* spp. approximately 100 grams of algae. The experiment was conducted over a period of six months, during which mussels were measured monthly, and water samples were collected for nutrient analysis, including ammonium, nitrite, Total Oxidise Nitrogen (Nitrate+Nitrite) TOxN, phosphate and silicate.

## Results

During the six-month experiment, mussels exhibited growth with high variability however, no statistically significant differences were observed between mussels-only treatments and mussels co-cultivated with *Ulva*. Most nutrient concentrations showed no significant differences between tanks, except for TOxN. levels which were statistically lower in the *Ulva*-only tanks as opposed to the mussel-only tanks (Figure 1). Differences in the TOxN level were noted between the cohabitation and monoculture conditions, but these differences were not statistically significant. These are preliminary non-parametric test and further investigation will be made as this test does not count for a nested structure, nor changes over time.

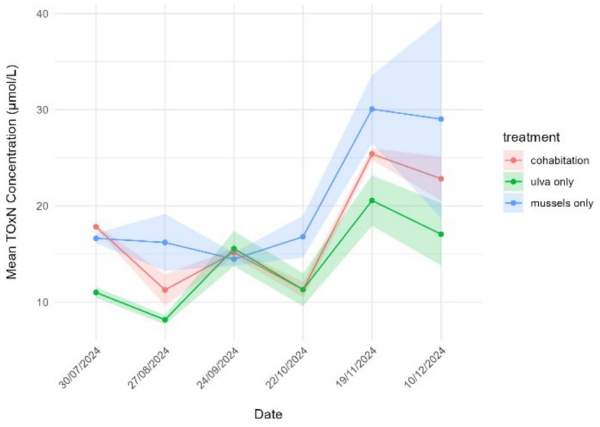


Figure 1: Mean concentration of TOxN overtime per treatment with standard error

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## Discussion

Although this study did not provide evidence of improved growth of mussel and *Ulva* when co-cultured, it highlighted the ability of *Ulva* to effectively uptake TOxN in the RAS setting. The lack of a significant enhancement in growth in the co-cultured tanks, in both species, may be the result of different factors including the practical challenges of managing both species within shared tanks. Maintaining optimal conditions for both organisms highlighted the logistical complexities of co-cultivation in a confined system. Therefore, a sequential tank setup, where mussels and *Ulva* are cultivated separately but interconnected (with water moving from mussels to *Ulva*), appears more feasible, and would likely maximise the nutrient uptake from *Ulva*. Stocking density of both species and water circulation within the RAS setups may also have influenced the interactions between mussels and *Ulva* in the co-cultured tanks.

Future research should involve scaling up experiments to open waters to evaluate potential growth differences under natural conditions, where hydrodynamic (current, wave action) can affect pseudo-faeces dispersal and nutrient uptake by seaweed. Key factors such as the stocking density of seaweed and mussels, size of the farm, proximity between seaweed and mussels, local hydrodynamic and environmental conditions (such as dissolved inorganic nutrient concentrations), should be investigated to determine the ecological interactions and operational feasibility of these integrated setups.

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# A NUTRITIONAL SOLUTION DECREASES SEA LICE ATTACHMENT IN ATLANTIC SALMON BY 70% IN CONTROLLED CONDITIONS IN VIVO

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## Introduction

Sea lice is one of the major problems for salmon farming operations, resulting in 2024 in over 3000 mechanical treatments that have the potential to negatively impact fish performance, fish welfare and farm benefits (Moldal et al., 2025). Industry efforts to decrease parasite pressure combine technical, medical and genetic solutions (Santigosa, 2025). Moreover, the use of nutritional approaches to optimize sea lice management is often considered as part of the toolbox. To demonstrate the benefits of a feed additive on sea lice attachment, in this trial we tested a control feed versus three experimental feeds containing a matrix-encapsulated phytogenic that is based on essential oils of oregano, thyme, and citrus (Digestarom®) at three inclusion levels (400, 800 and 1200 g/ton). The aim of the trial was to validate the following hypothesis: this feed additive can reduce the attachment of sea lice at adult stage whilst maintaining zootechnical performance.

## Methods

Atlantic salmon post smolt (186g) were acclimated for 7 days in seawater tanks and then fed one of the dietary trial treatments for 45 days, using triplicate tanks per treatment. After this pre-challenge period, animals were challenged with sea lice (*Lepeophtheirus salmonis*) using a flush. The trial continued for a further 53 days. At the end of the trial (day 105), sea lice counts were performed, in addition to zootechnical performance and welfare scoring. Samples were taken for the determination of blood biochemistry.

## Results

Results show a significant decrease (70%) in the total number of sea lice with the two highest inclusion doses (800 and 1200 g/t). In addition, in these two treatments there was a concomitant increase in the percentage of fish without any lice in those specific treatments. Feed intake was not negatively impacted by the inclusion of the feed additive. Final body weight, FCR and SGR welfare scores were similar across the four treatments.

## Conclusions

The role of feed additives in sea lice management has long been considered as part of the toolbox to reduce sea lice infestation in salmon farming operations. The results obtained in this trial show that the in-feed inclusion of Digesterom® at 800 and 1200g/ton had a significant effect on sea lice attachment, reducing the count by 70%. The findings from the present R&D work support the need for large-scale commercial testing of this feed additive as the next step toward full validation.

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# EFFECTS OF *Ulva ohnoi* INCLUSION IN DIETS OF PACIFIC WHITE SHRIMP POST-LARVAE REARED IN A BIOFLOC SYSTEM

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## Introduction

Nursery systems using biofloc technology have proven effective in boosting productivity, improving survival, and minimizing environmental impacts in shrimp farming. However, in subtropical regions, cold fronts during autumn and winter pose a growing challenge by lowering temperatures, which negatively affect shrimp health and growth. Recently, macroalgae have gained attention as feed additives due to their nutritional value and their immunostimulant, antiviral, antibacterial, and growth-enhancing properties, offering a sustainable alternative to antibiotics in shrimp aquaculture. This study aimed to assess the effects of including dry biomass of the green macroalga *Ulva ohnoi* in the diet of *Penaeus vannamei* post-larvae reared in a biofloc system, focusing on growth performance, post-larvae microbiology, and thermal shock resistance.

## Material and methods

The study was conducted at the Marine Shrimp Laboratory, Department of Aquaculture, Federal University of Santa Catarina, Brazil *U. ohnoi* was sourced from an integrated multitrophic aquaculture system using biofloc technology, included in diets at three levels (0, 0.5, and 2% inclusion) and added to diets to replace kaolin. Post-larvae, with an initial mean weight of $0.068 \pm 0.01$ g, were reared for 46 days in 60-L tanks at a density of 2,000 shrimp $m^{-3}$ and fed six times a day. The nutritional requirements of *P. monodon* were used as a reference for formulating diets for *P. vannamei*.

## Results

Temperature was kept at 28 °C and dissolved oxygen above 5 mg $L^{-1}$ in all treatments. No significant differences were observed among treatments for total ammonia nitrogen, nitrite, nitrate, alkalinity, pH, total suspended solids, or salinity. Shrimp fed with 0.5% of *U. ohnoi* in diet showed significantly higher final mean weight (an 18.7% increase), weight gain, and growth rate than those in the control group, without affecting survival or feed conversion ratio. Increasing the levels of *U. ohnoi* in the diets led to a rise in the abundance of heterotrophic bacteria in shrimp, with the 2% inclusion treatment showing a significantly higher bacterial count compared to control group. Although a numerical reduction in *Vibrio* spp. was observed in shrimp fed with algae-supplemented diets, this decrease was not statistically significant when compared to control. Additionally, the inclusion of *U. ohnoi* had no effect on the thermal resistance of shrimp reared in the biofloc system over the 46-day period.

## Conclusions

Considering shrimp performance was similar across diets containing *U. ohnoi*, the inclusion level of 0.5% is recommended, as it resulted in higher growth rates compared to control group.

## Acknowledgements

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## Note

This abstract summarizes the findings from the published article:

Santos, I. L., Carneiro, R. F. S., Morais, A. P. M., Martins, M. A., Seiffert, W. Q., & Vieira, F. N. (2024). *Ulva ohnoi* as a feed additive for Pacific white shrimp post-larvae cultured in a biofloc system. Aquaculture International, 32, 8809–8822. https://doi.org/10.1007/s10499-024-01591-4

# OPTIMIZING MICRODIET PERFORMANCE FOR FISH LARVAE: EFFECTS OF BINDERS AND TECHNICAL ADDITIVES IN FLUIDIZED BED AGGLOMERATION

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## Introduction

The production of formulated feed for fish larvae is crucial in aquaculture, as it directly affects larval survival, growth, and health. During early development, larvae have specific nutritional needs and limited feeding and digestive capacity, making the production of high-quality microdiets challenging. These diets must have precise physical and nutritional characteristics—such as suitable particle size, buoyancy, water stability, and digestibility—to ensure effective ingestion and nutrient absorption. Achieving these features is particularly difficult with conventional processing methods when particles below 0.5 mm are required (Pinto et al 2019).

Fluidized bed agglomeration (FB agglomeration) offers a promising solution. This technology transforms fine powders into granules with controlled size and improved physical properties, while preserving thermolabile compounds. In this process, particles are suspended in hot air and bound using a sprayed binder solution, producing agglomerates with defined size and density. Benefits include improved flowability, water stability, particle uniformity, and incorporation of sensitive ingredients (Dhanalakshmi et al., 2011). This enables the production of high-performance microdiets suited to the demanding needs of fish larvae.

This study evaluated the effects of different types and levels of binders and technical additives on the physical and functional properties of larval feeds. Three binder types—proteins, gums, and carbohydrates—and two technical additives were tested. Feed pellets were analyzed for water stability, nutrient retention, sinking rate, flowability, water dispersion, and larval acceptability.

## Materials and methods

All diets were produced at NIRO GEA MP1 (Denmark) under standardized conditions. The powders were placed in the expansion chamber and heated up to 40ºC. Subsequently, the oils were added by spraying, followed by the binder. After the binder was added, the product temperature was allowed to return to 40ºC before ending the process. The diets were then sieved into the following fractions: 0.1–0.2 mm, 0.2–0.4 mm, and 0.4–0.6 mm. To determine the physical properties of the diets, methodologies developed internally at SPAROS were used.

## Results

The protein leaching was around 10% for all binders tested, except for one non-protein binder, which showed a lower leaching value of approximately 6% and higher water stability. No significant differences were observed among prototypes in terms of bulk density, flowability, sinking behavior, or dispersion. The bulk density ranged between 320–380 g/L. All prototypes initially exhibited low flowability and a high sinking velocity. However, following the addition of the technical additives, an improvement in dispersion was observed, which indirectly led to a reduction in the sinking velocity of the pellets and an improvement in flowability behavior. The larvae acceptance tests are still ongoing.

## Discussion

The protein leaching values around 10% for most binders indicate a relatively moderate level of nutrient loss to the surrounding water, which is a critical parameter in larval feeding due to the sensitivity of larvae to nutrient availability. Notably, one of the non-protein binder showed a significantly lower leaching rate (~6%) and improved water stability, highlighting its potential to enhance pellet integrity and reduce nutrient wastage. This suggests that some binders may play a key role in improving microdiet performance, possibly by forming stronger or more cohesive matrices during agglomeration.

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During the first set of trials, the lack of significant variation in bulk density, flowability, and sinking behavior among the prototypes suggests that the agglomeration process was consistent and that the binder type alone may not drastically influence these parameters under the tested conditions. However, all diets showed low flowability and high sinking velocity, which could hinder feeding efficiency, particularly for surface or mid-water feeding larvae.

In a second set of trials, we observed improvement in dispersion and flowability after the addition of technical additives. These additives appear to have enhanced the physical behavior of the pellets, likely by modifying surface characteristics or water interaction dynamics. This is relevant as improved dispersion can increase the availability of feed in the water column, thereby enhancing feeding opportunities and potentially improving larval growth and survival.

Ongoing larvae acceptance tests will provide critical complementary data, as physical quality must ultimately align with biological performance. The integration of physical and biological results will be essential to identify the most effective formulation strategy for high-performance microdiets.

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# DIFFERENT COMBINATIONS OF MARINE ALGAE BIOREFINED EXTRACTS AS BOOSTING STRATEGIES TO IMPROVE EUROPEAN SEABASS *Dicentrarchus labrax* JUVENILES IMMUNITY

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## Introduction

The use of functional ingredients is considered a valuable tool to promote fish robustness and welfare during intensive farming. Among them, marine algae arise as promising candidates for inclusion in aquafeeds due to their antibacterial, immunestimulant and anti-oxidant properties. However, information regarding the potential of combining different algae biomasses and extracts as well as optimal supplementation periods is still lacking. Therefore, the present study aims to evaluate innate immune defenses and disease resistance of European seabass, *Dicentrarchus labrax*, juveniles fed diets containing different combinations of biorefined algae-derived compounds.

## Materials and methods

After acclimation, 1080 fish (12.12 ± 1.01 g) were randomly distributed in 15 tanks to which the experimental diets were assigned in triplicates. Dietary treatments consisted of a commercial diet (CTRL), a commercial diet with an immunestimulant (CTRL+) and three CTRL-based diets in which a mix of *Ulva* extract and different algae broken cells were included (Diet1 - *Ulva* spp., Diet2 - *Gracilaria gracilis*, Diet3 - *Euglena gracilis*). The boosting strategy consisted of feeding the animals for two different periods (5 and 14 days) before a stressfull stimulus. The chosen stressful condition was a bacterial challenge (intraperitoneal injection, 2.75 x $10^5$ CFU/mL of *Photobacterium damselae piscicida* strain MT1415). Samplings were performed immediately before and 6 hours after the bacterial challenge. At each sampling point, fish were euthanized (n=9 per tank) and sampled for blood (haematology), plasma (humoral immune defences), liver (oxidative stress), anterior intentine and head-kidney (gene expression).

## Results

The statistical analysis allowed a clear distinction between non infected and infected animals among groups within the same boosting strategy. For a better comprehension of diet-induced effect on fish immune status and response to infection, a discriminant analysis (DA) was performed for each sampling time. After 5 days of feeding, a clear separation between non-infected fish fed Diet3 and their counterparts fed Diet1, Diet2 and CTRL+ was observed, mainly driven by the positive load on expression of genes related with epithelial cells integrity and detoxifying enzymes in the anterior intestine. However, in infected groups, both Diet3 and Diet2 were significantly separated from those fed CTRL. This discrimination was positively loaded by the intestinal gene expression related with the antioxidant response and cell stability, in opposition to a negative loading of pro-inflammatory cytokines transcripts. The longer-term stimulation with algae derived compounds have produced no significant differences both before and after infection.

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## Discussion and conclusion

The experimental diets showed no significant immune modulation on both boosting strategies tested. However, Diet3 seems to have a beneficial effect on intestinal integrity after 5 days of feeding both before and after bacterial stimulus, once there was an increased gene expression levels related with cell adhesion.

Gathering all the information, it can be hypothesized that a short-term feeding period using a combination of *Ulva* spp. aqueous extract and *Euglena gracilis* broken cells (Diet3) may contribute to the stability and integrity of intestine, enabling an enhanced resistance to pathogenic threats. Future work will focus on microbiome and proteome analyses for a deeper comprehension of these diets' modulatory mechanisms.

## Acknowledgements

This work was financially supported by "Pacto da Bioeconomia azul" (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line "Agendas for Business Innovation" through the funding scheme C5 - Capitalization and Business Innovation. PS and AC were supported by Fundação para a Ciência e Tecnologia (2022.13449.BD and 2022.12761.BD respectively). MH was supported by Fundação para a Ciência e Tecnologia (2023.07575.CEECIND/CP2848/CT0014).

# ANTIMICROBIAL ACTIVITY OF ULVAN-RICH EXTRACTS FROM *Ulva spp*. OBTAINED BY AUTOHYDROLYSIS

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## Introduction

The green macroalgae *Ulva spp*., has attracted growing interest due to its content of bioactive molecules with potential applications in biotechnology and health. Notably, ulvans—sulfated polysaccharides present in its cell walls—have been associated with antioxidant, immunomodulatory, and antimicrobial activities.

Among the available extraction methods, autohydrolysis stands out as a sustainable alternative, relying solely on water and elevated temperature to release functional components from the biomass. This solvent-free approach yields complex extracts containing a mixture of compounds such as polysaccharides, proteins, phenolics, and minerals.

In the present study, we examined the effects of *Ulva* extracts obtained by autohydrolysis and ethanol purification on the growth kinetics of three clinically relevant bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. These microorganisms are frequently implicated in opportunistic and nosocomial infections, making them relevant models for evaluating the antimicrobial activity of algal-derived compounds.

## Materials and Methods

Fresh Ulva biomass, sustainably cultivated and manually harvested along the Galician coast under certified organic practices, was subjected to autohydrolysis at 160 °C. The resulting crude extract (AF) was then purified by ethanol precipitation, yielding a second extract (AFp) enriched in water-soluble compounds, including ulvan-type polysaccharides.

Both extracts were characterized to determine their physicochemical properties, including molecular weight distribution, ash content, protein levels, total phenolic content, antioxidant capacity, carbohydrate composition, and mineral profile.

The antimicrobial activity of the crude and purified extracts was evaluated against three clinically relevant bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. Growth kinetics were assessed in the presence of the extracts, using standard microplate assays to monitor optical density and calculate inhibition effects.

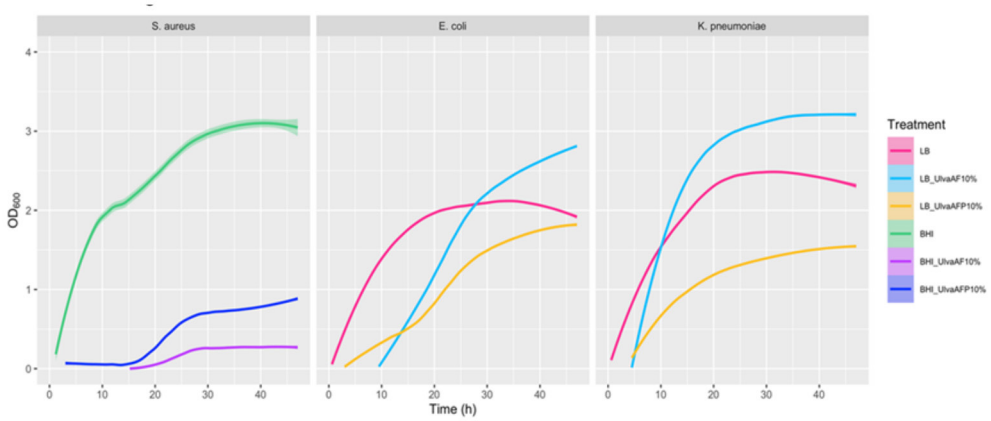


Figure 1. Growth kinetics of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* in the presence of crude (AF) and ulvan-enriched (AFp) Ulva extracts.

*(Continued on next page)*

**Results**

The growth inhibition assays demonstrated that both crude and purified Ulva extracts had a noticeable antimicrobial effect on *S. aureus*, with growth curves significantly below those of the control. Notably, the purified extract (AFp), which was enriched in ulvans, exhibited a prolonged lag phase and reduced final population density compared to the crude extract (AF), suggesting enhanced antimicrobial potency.

For *E. coli* and *K. pneumoniae*, similar trends were observed, although to a lesser extent, indicating a possible strain-dependent response to ulvan-rich extracts.

**Conclusion**

Our results demonstrate that Ulva extracts obtained through environmentally friendly autohydrolysis and ethanol purification possess the ability to modulate bacterial growth. These findings suggest that compounds present in the extracts may interfere with bacterial colonization mechanisms.

This study reinforces the value of macroalgae as a renewable source of multifunctional bioactives and supports the exploration of Ulva-derived products in the development of alternative microbial control strategies. The sustainable nature of the extraction process adds further relevance, aligning with current efforts to identify eco-conscious solutions with potential applications in aquaculture, food safety, and biomedical fields.

# MATURATION OF A RAS BIOFILTER IN A FISH LARVAL REARING FACILITY AT THE MARIEL MARICULTURE STATION, ARTEMISA PROVINCE, CUBA

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## Introduction

Recirculating Aquaculture Systems (RAS) reuse the maximum volume of water for the cultivation of aquatic organisms, maintaining water quality through various filtration methods. A RAS comprise production units equipped with automated feeding systems and sensors to control water chemical parameters such as pH and oxygen. It may also include mechanical and biological filtration systems, as well as UV-treatment for pathogen removal. Biological filtration includes the removal of nitrogen compounds as measured by *e.g.* Total Ammonia Nitrogen (TAN) from the water through nitrification performed by nitrifying bacteria as a biofilm on a carrier substrate with a large surface. The maturation of biofilters is the period when the nitrifying bacteria colonize the substrate. This process is a crucial step in the startup of a RAS system (Timmons *et al*., 2018; Pulkkinen, 2020; Balami, 2021; Burut-Archanai *et al*., 2021). A biofilter is considered mature when the concentration of TAN stabilizes below 0.7 mg/l (Rojas & Pedersen, 2016). As an outcome of the Cuban/Norwegian project "Production of marine fingerlings in Cuba", a RAS have been installed at the Mariel Mariculture Station of the Centro de Investigaciones Pesqueras (CIP). This study describes the maturation process that is observed in the biofilter of the larval rearing area of the Mariel Station, Artemisa Province, Cuba.

## Materials and methods

The maturation process of the RAS biofilter for tilapia production, were initiated the medio February 2025. Sediments from culture tanks of tilapia within the culture units of the area, were applied as fertilisers. During maturation, temperature and salinity were measured daily. The pH and TAN were measured once a week applying the commercial kit Colombo Aquatest (Colombo, The Netherlands), with a lover level of quantification for $NH_3$ of 0.5 mg/L. Nitrite, nitrate and phosphate were also measured on a weekly basis by the same kit, and with quantification limits of 0.5, 10.0 and 0.5 mg/L, respectively. In addition, water samples were collected from the culture units and biofilter carriers twice a month, for the subsequent identification of the bacterial community and its succession.

Furthermore, a laboratory experiment was conducted simulating RAS conditions with the aim of identifying the salinity range in which the biofilter maturation process occurs most efficiently. This experiment aimed to optimize the biofilter maturation process that will be carried out in the breeding and weaning areas of the RAS system in Mariel Mariculture Station.

In the laboratory experiment, three ranges of salinity were evaluated, each in hree replicates: Treatment 1 (T1): 30-40 ppm; Treatment 2 (T2): 20-30 ppm; Treatment 3 (T3): 10-20 ppm. Each replicate consisted of 10 L aquarium where biofilter particles were added and that were daily fertilized with water from siphoning two 60 L tanks with 20 tilapia (weighing 2-4 g each). The experiment began on the 6th of February and ended on the 12th of March 2025. During the first week, 100 mL of combined siphoning water from both fish tanks was added to all replicates. The volume was increased to 200 mL in week 2, and since week 3, 300 mL were added. Temperature and salinity were measured daily. Nitrite, nitrate, phosphate, ammonium and pH were measured once a week. In the last week of the experiment, water samples were taken from all the replicates and from the fish tanks, and biofilter carriers were sampled from all replicates to determine significant differences between the bacterial communities. The analysis of bacterial communities will be conducted in two parts, one through classical taxonomy at the lab of CIP in Cuba and the other through DNA sequencing at the Institute of Marine Research (IMR), Bergen, Norway.

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**Results**

The TAN values were less than 0.5 mg/L during the entire period the values were measured. This happened in the Mariel Station and during the experiment conducted in the lab. Nitrite values went from 0.5 mg/L during four weeks at the Station to a spike in week 5, presenting 2 mg/L during three consecutive weeks. Something similar occurred with nitrate, going from 20 mg/L to 50 mg/L in the same period. With the aim of keeping the welfare of the fish, the density of the culture was decreased, and the nitrite and nitrate values were restored in week 8.

**Conclusions**

A TAN value lower than 0.7 mg/L, are the main indicator of a mature biofilter. Given that the values of these compounds were less than 0.5 mg/L from the beginning in both experiments, at the Station and the lab, the nitrite and nitrate were taken as the main indicators. The increase in the values of both compounds during week 5 until week 7 in the Station may be due to the development of the nitrifying bacteria that carry out the first step of the nitrification process (ammonium oxidation). Nonetheless, the results of the bacterial community identification from the samples that were taken during the entire experiment, will allow a better understanding of the functioning of the biofilter and to give definitive conclusions about the experiment. The above is also the case in the experiment conducted in the lab, where the bacterial community analysis to be conducted is necessary to determine if there were significant differences between the salinities examined.

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# ADVANCES IN THE QUANTIFICATION OF NATURAL ANTIOXIDANTS (NAOX) IN FISH FEED INGREDIENTS BY LC-MS AND CORRELATION WITH ANTIOXIDANT ACTIVITY

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## Introduction[1]

Oxidative stress is a widespread condition generated by various environmental and farming practices. leading to poor fish health and quality issues. Insufficient dietary antioxidants to alleviate oxidative stress have generated the need for more natural alternatives, and several promising natural antioxidants (NAOX) have been identified. For their successful application in aquaculture, one critical factor is the use of reliable and accurate methods for NAOX quantification in the production chain, that allow a better study of their effects, more controlled processes, and higher reproducibility in the production conditions. Liquid chromatography mass spectrometry (LCMS) is commonly used for analysing from few to over hundreds NAOX in diverse types of samples. However, aspects such as the stability of the compounds and matrix effects are often ignored although they can have an important impact on the accuracy of the results. Thus, in this work, we implemented a LC/MS method for accurate and reliable quantification of NAOX in a commercial antioxidant ingredient containing natural sources commonly used.

## Materials and Methods

Three commercial samples of antioxidant ingredients for fish feed, containing a mixture of NAOX, were supplied by Arctic Feed Ingredients (AFI, Steinskjer, Norway). An accurate and reliable method by LC-MS illustrated in Figure 1. was implemented for quantification of the main NAOX. The comprehensive study included; 1) the evaluation of different solvents for extraction of the NAOX from the samples, 2) optimization of the instrumental parameters for the collision cell in mass spectrometry, and 3) stability studies of the NAOX in the selected solvent individually and in groups. For the latter, the stability was measured as the change of the LC-MS signals of the polyphenols in terms of their storage time (0, 1, 6, and 9 days), temperature (4, – 20, and – 80 °C), vial colour (transparent, amber), and concentration (0.1 and 30 $\mu$g/mL). The analytical method was validated for selectivity, linearity, detection, and quantification limits (LOD and LOQ) and additionally, a comprehensive study of matrix effects was included. As evaluation of the antioxidant capacity, CUPRAC and DPPH methods were used.

## Results and discussion

Methanol showed the global best performance for extraction for all the selected compounds in the evaluated solvent. Regarding stability of the LC-MS signal of the NAOX, no consistent effect was observed for the storage of the solutions at different temperatures, or the different vials, but in contrast, time of storage was found to affect the signal obtained. In general, a reduction of the signals was observed for 3 out of 6 NAOX. The worst stability was found for 2 NAOX, for which even 1 day post preparation, changes were more noticeable than for the other compounds. Stability improved with higher concentrations of the NAOX.

For the validation parameters, evaluation of the extracted ion chromatograms demonstrated that LC-MS/MS can determine the selected NAOX without interference from other components in the sample. The matrix effect, evaluated not in a single point but by comparison of the slopes of external standard and standard addition calibrations for each compound, was negligible for 3 out of 6 compounds. However, significant matrix effects were observed in the other 3. For instance, the ratio of the standard addition to external standard slope for 2 NAOX showed enhanced sensitivity of 3.84 and 1.93, respectively. In contrast, the same relationship for a 3$^{rd}$ one was 0.47, indicating a significant signal suppression of the sample matrix on the analytical signal. Considering the important matrix effects found, the linearity was assessed by using standard addition calibrations by coefficient of regression ($r^2$) and a lack of fit test using the experimental Fisher test for the 6 compounds. The six models were

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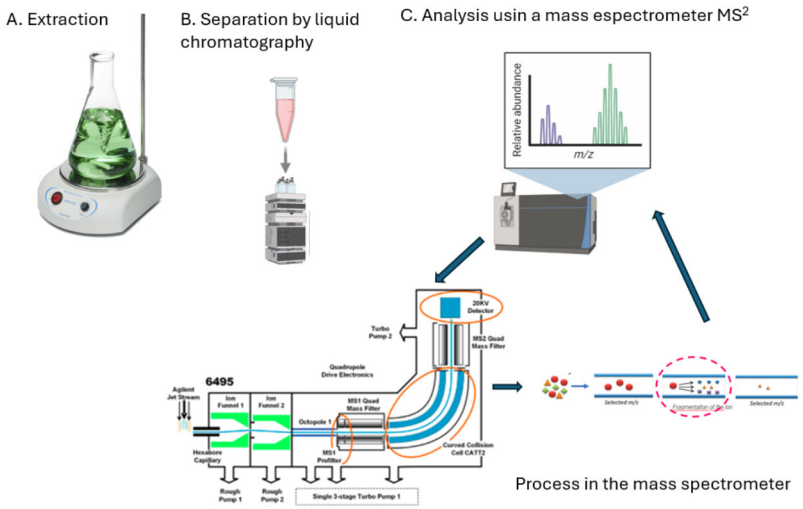


**Figure 1.** LC-MS analysis applied to the quantification of NAOX in commercial antioxidant ingredient samples

used to evaluate LOD, LOQ, and method accuracy by introducing the experimental signals back into the calibration model to determine the estimated concentration in relation to the nominal value. The accuracy for the six polyphenols was within the ± 15 % interval of variation suggested by the ICH and regarded as acceptable for the determination of polyphenols in fish feed ingredients.

The antioxidant activity was evaluated by 2 different methods, CUPRAC and DPPH. The total content of polyphenols quantified in the samples did not show a direct correlation with the antioxidant capacity. Possible explanations are the different potency of individual compounds and/or the presence of NAOX not quantified.

Conclusion

To the authors knowledge, this is the first report on the validation of a LC-MS/MS method for quantifying selected polyphenols in commercial fish feed ingredients, supplemented with polyphenols from plant extracts using the standard addition method. The study highlighted the importance of performing a prior evaluation of the stability and matrix effects to decide the correct calibration strategy. The method showed adequate performance and demonstrated to be adequate for the accurate quantification of NAOX in industrial samples in aquaculture. The complementary study of antioxidant capacity showed that both approaches are complementary and important to product applications and standardizations.

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# NATURAL ANTIOXIDANTS FROM INGREDIENT TO FISH

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## Introduction

The use of natural antioxidants (NAOX) for preventing fish feed oxidation and reducing oxidative stress in fish is gaining popularity. NAOX present a more sustainable and consumer acceptable alternative to the more traditional, synthetic antioxidants. Further, NAOX contribute to the substitution, or partial replacement of synthetic antioxidants, which is especially relevant following the restriction of ethoxyquin and the anticipated reduction of BHT and BHA.

In order to implement NAOX effectively, it is imperative to study the behaviour of those compounds with natural antioxidant activity throughout the whole production cycle, from raw materials, through the stages of feed production, to the fish tissues. This project studied the effects of a commercial NAOX ingredient that was added to a commercial fish feed at two levels (0.01 and 0.05%). The objective was to analyze quantitatively selected antioxidant compounds (polyphenols) in various stages of a fish farming process for salmon. Extraction methods for the selected polyphenols were developed for different matrices: antioxidant ingredient, fish feed, and fish tissue and each matrices was analysed by LC-MS/MS. Additionally, for studying the antioxidant effect of the NAOX throughout the production process, several oxidation and oxidative stress indicators were measured; in feed ingredients, in feed at different stages of production, in feed after 3 months storage, and in the tissues of fish fed the two diets.

## Materials and Methods

The experimental diets were formulated to meet the nutritional requirements of Atlantic salmon and were produced at the BioMar AS pilot plant. A commercial antioxidant was added at different inclusion levels (0.0, 0.01 and 0.05%). The fish trials were conducted at Matre Aquaculture Station, Institute of Marine Research, Norway. Nine tanks (1×1×0.4 m3) were stocked with 35 post-smolts with an average weight of 473±11 g. Fish in triplicate tanks were randomly assigned to each of the three diets.

The antioxidant ingredient used in the production of the NAOX diet was sampled for analysis. Samples of the three fish feed meals used in the fish trial were also taken before and after extrusion, and after drying (final feed). Several parameters related to feed quality were measured: dry matter, protein, vitamins C and E, astaxanthin, TBARS, total (tGSH) and oxidized (GSSG) glutathione, peroxide value (PV) and anisidine value (AV). Additionally, a LC-MS method established previously was used for the quantitative analysis of the main NAOX compounds in the antioxidant ingredient, in different stages of the production of the 3 diets with different inclusion levels of NAOX (0.0, 0.01 and 0.05%) and in tissue (liver and muscle) of the fish fed the 3 diets.

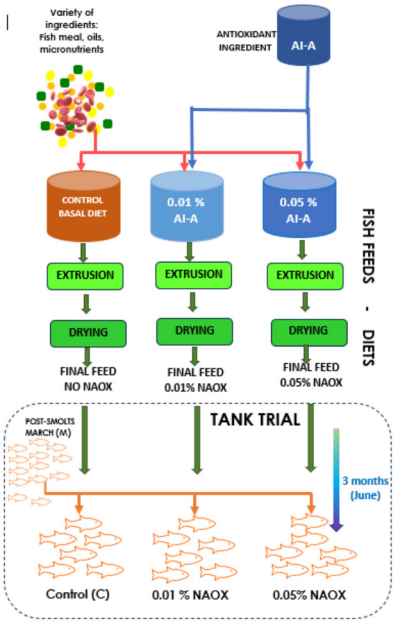


**Figure 1.** General scheme of the study

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For the quantitative analysis of the selected NAOX compounds (polyphenols), extraction of the antioxidant ingredient and the fish feed samples containing different levels of the commercial NAOX ingredient (0, 0.01, 0.05 %) were carried out, as well as at different stages of production (before extrusion, before drying and final feed).

In sampled fish, growth, condition factor, body indices, and cataracts were recorded. Liver and muscle samples were collected and pooled from three fish per tank and analysed for vitamin C, vitamin E, MDA, and astaxanthin. Individual liver and muscle samples from an additional three fish were analysed for GSH/GSSG and antioxidant enzyme analyses. Plasma was taken from all 9 fish and pooled before analysis of vitamin C and vitamin E.

**Conclusions**

The main NAOX polyphenols were quantified in fish feed ingredients, fish diets at different stages of production, and fish tissues, allowing the complete traceability throughout the production process. The NAOX content was not stable, an important loss of polyphenols was evidenced, especially during the extrusion process. However, positive results were observed in feed, lower TBARS and a protection for tocopherol were observed, as well as a possible protection of astaxanthin.

The analysis of liver and muscle samples, dissected from salmon fed the final feed of the production process, revealed that two polyphenols were transferred into these tissues. Interestingly, and despite of the loss of NAOX during the production of the feed, results from oxidative parameters suggest that dietary NAOX affected MDA and glutathione metabolism in both the muscle and liver of Atlantic salmon: MDA was lowered at 0.01% NAOX in the muscle, while 0.05% was necessary to significantly lower liver MDA. This could indicate that dietary NAOX prevented *in vivo* lipid oxidation in the fish tissues. GSH and GSSG were lowered in the liver while Eh was constant. In the muscle, GSH increased while GSSG was constant, leading to a more reduced Eh. This shows that redox metabolism was affected by dietary NAOX. No effects of the dietary NAOX were observed on growth, condition factor, body indices or cataracts. The results obtained were promising and further studies are needed for advancing in the understanding of NAOX in redox biology of fish.

**NOTE:** This work is in parallel/relates to the methods developed and described in the first abstract submitted: 1_Sarmiento et al_ V3: ADVANCES IN THE QUANTIFICATION OF NATURAL ANTIOXIDANTS (NAOX) IN FISH FEED INGREDIENTS BY LC-MS AND CORRELATION WITH ANTIOXIDANT ACTIVITY

# IMPACT OF ALTERNATIVE FEED INGREDIENTS AND FEEDING STRATEGIES ON MUSCLE MORPHOLOGY, AND FILLET QUALITY OF GENETICALLY SELECTED GILTHEAD SEABREAM (*Sparus aurata*) IN A LONG-TERM FEEDING TRIAL

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**Introduction**

To address the limited availability of fishmeal (FM) and fish oil (FO), it is necessary to find new sustainable ingredients, such as animal by-products, insect meal, and microalgae oil, with proven benefits in aquafeeds (Carvalho *et al.,* 2024). Additionally, restricted feeding strategies reduce costs and improve sustainability by minimizing feed waste and enhancing water quality (Assis *et al*., 2020), while selective breeding boosts growth and diet adaptation (Carvalho *et al*., 2024). However, these strategies can affect fillet texture and shelf life, influencing consumer acceptance. Understanding post-harvest proteolytic degradation and muscle changes is essential for preserving fillet quality (Castro *et al*., 2022). This study evaluates the combined effect of sustainable alternative diets, genetic selection, and feed restriction strategies on muscle histomorphology and fillet texture of gilthead seabream (*Sparus aurata*; GSB) over a long-term feeding trial up to commercial size, with additional attention to fillet quality during shelf life.

**Materials & Methods**

GSBs selected for high growth (HG) or non-selected (REF) were reared for 300 days until apparent satiety (AS) or with food restrictions (85% or 65% AS) using a control diet (CON) with low FM and FO balanced with vegetable meals (VM), and an alternative diet (ALT) where FM was half-replaced with insect and animal by-product meals, while FO was completely replaced by microalgae and animal by-product oils.

Texture profile analysis was performed at 1 and 4 days post-harvest (dph) using a TA.XT2 Texture Analyzer. Muscle samples from 1 and 4 dph were paraffin embedded, sectioned, and analyzed by immunohistochemistry for calpastatin, calpains, and actin, and evaluated blindy. Immunostained sections were scanned and semi-quantitatively analyzed. Muscle ultrastructure was processed for Transmission Electron Microscopy (TEM; SIMACE ULPGC) to assess mitochondrial, sarcomere, and connective tissue integrity.

**Results**

Fillets from fish fed the ALT diet showed significantly higher hardness and adhesiveness than those fed the CON diet, while REF fish exhibited greater fillet cohesion, gumminess and resilience than HG fish. The 85%AS feeding strategy led to the best overall texture. Ice storage reduced all texture parameters after 4 dph.

No major histological differences were observed at 1 dph. At 4dph, actin and m-calpain reactivity increased and a slight decrease in fiber-to-fiber adhesion and detachment to the myocommata was noted. TEM analysis revealed that 65%AS feed restriction caused mitochondrial elongation and fusion. Ice storage led to ultrastructural degradation, including mitochondrial damage, loss of sarcomere integrity (decrease Z-disc integrity and increase I-band width), and interfibrillar disjunction.

**Discussion**

The ALT diet improved hardness via higher hydroxyproline from animal by-products compared to VM, strengthening connective tissue (Donadelli *et al*., 2006). HG fish, with greater hypertrophy, had softer texture due to lower fiber density (Palstra *et al*., 2011). Feeding strategy had the greatest impact on fillet texture. Compared to AS, moderate feed restriction (85%AS) increased firmness by reducing fat content, as high fat softens flesh (Lefevre *et al*., 2015). Ultrastructurally, 65%AS feeding showed mitochondrial elongation, suggesting mitophagy and fusion under nutritional stress. This result, first described in fish, alings with studies in other species under starvation (Rambold *et al*., 2011).

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During ice storage, texture parameters declined alongside increased actin and m-calpain immunoreactivity, suggesting early calpain-mediated degradation (Ahmed *et al*., 2015). Calpain activity degrades actin filaments, disrupting Z-discs and increasing actin detection by antibodies, which reduces fillet firmness, affecting consumer acceptance (Singh and Benjakul, 2018). Ultrastructural changes, more evident at 4 dph, included mitochondrial dense granule formation and membrane rupture, linked to calcium accumulation, mitochondrial permeability, and activation of calpains proteases, which degrade structural proteins and soften flesh (Ahmed *et al*., 2015). Slight connective tissue detachment and interfibrillar separation appeared at 1 dph, progressing to marked Z-disc loss and increased I-band width by 4 dph, consistent with previous studies in GSB (Ayala *et al*., 2010).

**Conclusion**

Combining moderate dietary restriction and innovative diets can improve GSB aquaculture sustainability and product quality during long-term feeding. The ALT diet improved fillet texture. The 85%AS feeding strategy optimized fillet quality by preventing lipid accumulation and muscle adaptations, as observed with the other feeding strategies. Histological analyses were key to understanding postmortem muscle changes, offering new insight into supporting nutritional strategies to maintain GSB fillet quality.

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# EFFECTS OF ALTERNATIVE DIETS, GENOTYPE, AND FEEDING LEVEL ON INTESTINAL MORPHOLOGY OF GILTHEAD SEABREAM (*Sparus aurata*)

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## Introduction

Fishmeal and fish oil (FMFO), obtained from intensive fishing, remain key aquafeed ingredients, but their replacement with plant-based alternatives (VMVO) has shown negative effects on gut health in several carnivorous fish species (Montero *et al*., 2023). Sustainable alternatives such as insects, microalgae, and animal by-products are being explored for their high nutritional value. Additionally, restricted feeding strategies reduce costs and enhance sustainability by minimizing feed waste and improving water quality, while selective breeding promotes growth and dietary adaptation. However, knowledge of their combined effects is limited, and responses appear species- and context-specific (Montero *et al*., 2023). Alternative protein sources can affect intestinal morphology, including villus length, goblet cell number, and inflammatory cell infiltration (Gai *et al*., 2023). This study assesses the long-term combined effects of novel feed formulations replacing FMFO and VMVO, genetic selection, and feeding strategies on gut morphology and health in gilthead seabream (*Sparus aurata*; GSB).

## Material & Methods

GSBs selected for high growth (HG) or non-selected (REF) were reared for 300 days until apparent satiety (AS) or with food restrictions (85% or 65% AS) using a control diet (CON) with low FMFO balanced with VMVO; and an alternative diet (ALT) where FM was half-replaced with insect (IM) and animal by-product meals, while FO was completely replaced by microalgae and animal by-product oils. At the end of the trial, samples from the foregut and hindgut were collected, processed, sectioned, and stained with Alcian Blue–PAS. Sections were scanned and morphometric analysis performed using Image-Pro Plus software to measure lamina propria (LP) width and villus width and length. Goblet cell (GC) area was quantified automatically using the eyedropper tool within each fold. GC density was expressed as the number of cells per 1,000 μm² of villus area to account for villus size variability. Additionally, foregut villus tip detachment and fusion were assessed using a continuous intensity scale from 0 to 100.

## Results

Regarding foregut morphometry, villus width and length were not significantly affected by the different experimental factors. However, feeding the CON diet was associated with greater LP width. GSB fed the ALT diet showed a higher degree of fusion. Furthermore, HG fishes had less villus tip detachment than REF. Regarding GC, fish fed the CON diet had a higher intestinal GC density than those fed the ALT diet.

In the hindgut, HG fishes had a significantly greater LP width than REF. The highest food restriction (65%AS) decreased LP thickening. Furthermore, GSB fed the ALT diet showed significantly greater villi length than those fed the CON diet, while no significant differences were found in GC density per fold.

## Discussion

Regarding foregut morphometry, the CON diet was associated with greater LP width, irrespective of genotype, consistent with Randazzo *et al*. (2021), who reported increased LP in GSB fed VM compared with diets containing IM and poultry by-product meals. Foregut GC density per fold was also higher in the CON diet, aligning with studies in salmonids linking VM, particularly soybean meal, to elevated GC numbers, LP widening, and other enteritis-related changes (Seibel *et al*., 2022). Plant-based ingredients may promote inflammatory cell infiltration, thereby widening LP (Torrecillas *et al*., 2019). GSB fed the ALT diet showed a higher degree of fold fusion, consistent with previous findings where FM was partially replaced by IM (Gai et al., 2023). The greater fold tip detachment and stronger response of REF fish to dietary and feeding strategy changes suggest that HG fish exhibit greater intestinal plasticity, as previously reported (Montero *et al.,* 2023).

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In the hindgut, villus height was greater in fish fed the ALT diet, potentially enhancing digestive capacity, whereas higher VM levels have been associated with villus shortening (Seibel *et al.,* 2022). The 65%AS restricted feeding reduced LP thickening, likely due to the absence of feeding stimulation, as observed in fasted fishes (Nogueira Farias *et al.,* 2025). GC density per fold showed no significant differences, consistent with Di Rosa et al. (2023), who reported limited diet-induced changes in the hindgut compared with the foregut, likely due to nutrient absorption occurring primarily in the foregut.

**Conclusion**

Replacing FMFO and VMVO with a combination of novel alternative ingredients, modulated intestinal morphology in GSB, with responses differing by intestinal segment, genotype, and feeding regime. In the foregut, the CON diet (plant-based) increased LP width and goblet cell density and HG fish exhibited reduced villus tip detachment, suggesting higher intestinal plasticity. In the hindgut, the ALT diet increased villus length, while 65% feed restriction reduced LP thickening. These findings confirm region-specific intestinal responses, with the foregut being more sensitive to dietary modifications.

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# MUSHROOM INDUSTRY BY-PRODUCTS AS AN ALTERNATIVE INGREDIENT TO PLANT-BASED INGREDIENTS FOR RAINBOW TROUT (*Oncorhynchus mykiss*) AQUAFEEDS

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## Introduction

The aquafeed sector is progressively shifting towards reducing dependence on finite and costly marine-derived fish meal by diversifying formulations using plant-based ingredients (Naylor et al., 2021; Aragão et al., 2022). However, plant-based ingredients, particularly soybean meal (SBM), present several limitations, including reduced diet palatability, impaired fish growth performance and health, and escalating costs driven by their high demand across various animal and food production sectors, thereby casting doubt on their long-term sustainability (Zlaugotne et al., 2022). To overcome these challenges, continued efforts are required to identify novel, nutrient-rich, economically viable, and eco-friendly protein sources for the sustainable development of the sector (Naylor et al., 2021). One promising alternative protein source is mushroom by-products, often classified as waste, representing *ca.* 5% to 50% of the total mushroom biomass produced (Navarro-Simarro et al., 2024; Infochampi, 2025). These by-products have a rich nutrient profile, low cost, and excellent digestibility (Liu et al., 2025). Their valorization as aquafeed ingredients addresses both waste management challenges in the mushroom sector and the nutritional needs of aquaculture, aligning with a circular economy framework. Therefore, the present study aimed to evaluate mushroom industry by-products derived from *Agaricus bisporus* (AB) and *Pleurotus ostreatus* (PO) as a potential alternative to plant-based ingredients for rainbow trout (*Oncorhynchus mykiss*) diets.

## Materials and methods

Five isonitrogenous (47.21 ± 0.93%), isolipidic (24.34 ± 1.50%), and isoenergetic (5611.83 ± 39.11 cal/g) diets were formulated by replacing SBM at 0%, 41.67% and 83.33% with AB or PO meals (dried and ground biomass, CTICH, Autol, Spain), corresponding to nominal dietary inclusion levels of 5% and 10%. The diets were named according to the dietary inclusion levels and/or the type of mushroom as follows: D1 (control diet), D2 (AB5), D3 (AB10), D4 (PO5) and D5 (PO10). Diets were fed to rainbow trout (initial body weight: 22.07 ± 0.05g) *ad libitum* in quadruplicate tanks for 60 days. During the trial, feces were collected using a settling column to evaluate the apparent digestibility coefficients (ADCs) of the experimental diets. After 60 days, all fish were individually measured, and fish key performance indicators associated with growth performance, survival and feed efficiency were analyzed. Following fish biometry, four fish/tank (n=16/diet) were anesthetized (100 mg MS-222/L) for blood sample collection and biochemical and hematological analyses, while the rest were transferred to IRTA's biosafety level 2 challenge room for an *in vivo* bacterial challenge against *Aeromonas salmonicida* subsp. *salmonicida*. After determining the lethal dose 60 (LD60), fish (n = 10 fish/tank, 5 tanks/diet) were injected intraperitoneally with 0.1 ml of 2.43 x $10^6$ CFU/ml of *A. salmonicida* subsp. *salmonicida* and mortality patterns were monitored for 21 days.

## Results

At the end of the trial, no significant differences ($p > 0.05$) in survival were found among dietary groups. The ADCs of the experimental diets were high (crude protein and lipid ADCs > 91%), although the AB diets showed significantly lower crude protein ADC ($p < 0.05$) than the control diet. Fish fed the AB5 diet presented similar growth ($p > 0.05$) to fish fed the control diet, while fish fed the AB10, PO5 and PO10 diets presented lower performance ($p < 0.05$) (Figure 1). Fish fed the AB diets presented similar feed intake (FI), feed conversion ratio (FCR) and somatic indices (hepatosomatic, HSI; visceral, VSI; and perivisceral fat, PVFI indices) ($p > 0.05$) in relation to those fed the CTRL diet. However, fish fed the PO diets presented significantly reduced FI, FCR and HIS ($p < 0.05$), while VSI and PVFI remained unaffected ($p > 0.05$), compared to the group fed the control diet Significantly higher protein and lipid efficiency ratios ($p < 0.05$) were recorded in the AB5-fed group

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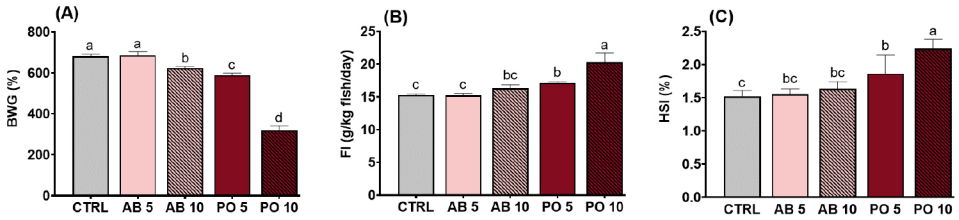


Figure 1. (A) Growth (% body weight gain, BWG), (B) feed intake (FI), (C) hepatosomatic index (HSI) in rainbow trout fed with the experimental diets containing different sources of mushroom meals for 60 days. Feed conversion ratio (FCR) followed the same pattern with FI (data not shown). Data are shown as mean ± SD (n = 4). Different letters indicate significant differences (ANOVA, $p < 0.05$). AB 5 (5% *Agaricus bisporus)*, AB 10 (10% *Agaricus bisporus)*, PO 5 (5 % *Pleurotus ostreatus),* PO 10 (10 % *Pleurotus ostreatus).*

compared to the rest of the dietary treatments. Blood counts and levels of ions and electrolytes, enzymes and metabolic and nutritional markers were similar for all experimental groups ($p > 0.05$), except for a significantly lower mean corpuscular hemoglobin, alkaline phosphatase, and sodium levels ($p < 0.05$) in fish fed the PO diets, particularly those fed the PO10 diet. The *in vivo* bacterial challenge results showed no significant differences in survival among dietary groups ($p > 0.05$).

## Conclusions

In conclusion, AB meal may replace plant-based diets, such as soybean meal, without compromising growth, feed efficiency, blood biochemical and hematological parameters, and the host's disease resistance, whereas the use of PO reduced fish performance.

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# MUSHROOM MEAL AS AN ALTERNATIVE AQUAFEED PROTEIN INGREDIENT: IMPACT ON GROWTH PERFORMANCE AND GUT MICROBIOME OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

Mushrooms are emerging as an eco-friendly alternative protein source due to their ability to convert organic waste, low production costs, and excellent digestibility (Liu et al., 2025). They are naturally rich in nutrients, particularly carbohydrates (*ca.* 35–70% dry weight), including complex cell wall polysaccharides such as chitin, β-glucans, and mannans. These compounds are recognized as "biological response modifiers," also referred to as prebiotics, due to their ability to modulate gut microbiota (Zhao et al., 2023). The gut microbiota plays a crucial role in numerous host biological processes, including digestion, absorption, metabolism of nutrients, and immune response, which together may contribute to promoting fish growth performance and health status (Vargas-Albores et al., 2021). For this reason, mushroom meal may be a promising aquafeed ingredient, not only as an alternative protein source but also for its potential functional effects, which may promote fish health status. Therefore, the present study formulated mushroom meal-rich diets derived from *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus* by-products and investigated their effects on the growth and gut microbiota of rainbow trout (*Oncorhynchus mykiss*).

## Materials and methods

Three experimental mushroom-based diets derived from *A. bisporus* (AB), *P. ostreatus* (PO), and *L. edodes* (LE) were formulated based on a control diet (CTRL) containing 49% crude protein and 22% crude fat. Mushroom meals (30%) were prepared by mixing with the CTRL diet (70%) and fed to rainbow trout juveniles (initial body weight: 120.1 ± 0.4 g) for 42 days. After 42 days, fish biometry was performed to evaluate growth, and representative fish samples (n = 4 fish/tank, 12 fish/diet) were euthanized, eviscerated, and their gastrointestinal tracts dissected for microbiota analysis. Gut samples were divided into three segments (pyloric caeca, PC; anterior intestine, AI; and posterior intestine, PI), and microbiota analysis was performed in each region. After DNA extraction, quality was checked, and the bacterial V3-V4 region of the 16s rRNA gene was amplified and sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end; FISABIO, Valencia, Spain). Bioinformatic analysis was performed using QIIME2 and DADA2 for cleaning and denoising, and taxonomic assignment with the SILVA database. Diversity and relative abundance were calculated based on the *microeco* package.

## Results

Results showed no significant differences in final fish body weight, body weight gain, and specific growth rate between fish fed the CTRL and mushroom-based diets ($p > 0.05$). Similarly, somatic indices (condition factor, hepatosomatic index, viscerosomatic index, and perivisceral fat index) and feed efficiency parameters (feed intake and feed conversion ratio) were not affected by the dietary inclusion of mushroom meals compared to fish fed the CTRL diet ($p > 0.05$).

In terms of microbial diversity across gut regions, alpha and beta diversity showed a stronger similarity between PC and AI than the PI. In addition, phylogenetic diversity, which considers the number of species and the phylogenetic distance, indicates that each region is different. In terms of composition, the three gut regions analyzed in this study were dominated by Firmicutes and Proteobacteria. Regarding the effect of diets, the AB-fed group showed reduced alpha diversity in the PC region compared to the group fed the CTRL diet ($p < 0.05$), whereas the alpha diversity of fish fed the LE and PO diets remained unchanged ($p > 0.05$). In terms of beta diversity, fish fed AB and LE diets presented significantly different bacterial community structures

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compared to the CTRL diet-fed group ($p < 0.05$). In the AI region, a similar pattern was observed, with fish fed the AB diet showing reduced alpha diversity compared to the CTRL-fed group ($p < 0.05$), whereas PO and LE fed groups were similar to those fed the CTRL diet ($p > 0.05$). Beta diversity showed significantly different structure among the CTRL, LE, and PO groups ($p < 0.05$). In the PI region, the alpha diversity of AB and LE was similar to the CTRL fed group, whereas this was reduced in the LE fed group ($p < 0.05$). Beta diversity showed that all dietary groups had significantly different bacterial community structure ($p < 0.05$).

In terms of microbial composition, the PC region showed that the CTRL-fed group has a higher relative abundance of Desulfovibrionaceae and *Ligilactobacillus* than the mushroom-fed groups ($p < 0.05$). Fish fed with AB showed a higher abundance of *Mycoplasma*, whereas the LE and PO groups showed dominance of Bacilli (class) and Bacillaceae *and Paenobacillus*, respectively. In the AI region, microbial composition for the CTRL fed group was dominated by Desulfovibrionaceae*,* Lactobacillaceae and *Weissella*. The AB-fed group showed a higher abundance of *Mycoplasma,* Bacilli (class) and *Legionella.* The LE-fed group did not reveal any significant change in bacterial abundance, whereas the PO-fed group showed an increase in *Ligilactobacillus* and Alphaproteobacteria. In the PI region, the CTRL-fed group maintained a higher abundance of Desulfovibrionaceae. The AB-fed group showed a higher abundance of Lactobacillaceae, whereas the PO-fed group did not show any significant change in bacterial abundance.

## Conclusions

Overall, the results showed that fish fed with mushroom-based diets presented similar performance to fish fed the control diet, suggesting that mushrooms may be a suitable alternative protein source in aquafeed. Furthermore, dietary inclusion of mushroom meals differentially modulated gut microbiota in terms of diversity and composition depending on the type of mushroom and region of the intestine considered. In particular, mushroom-based diets reduced the abundance of Desulfovibrionaceae across gut regions and shifted to an increase in different bacterial genera, particularly those that are classified as probiotics, such as *Lactobacillus,* indicating the potential of mushrooms not just as a protein source but also as a prebiotic ingredient.

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# ASSESSMENT OF THE GENETIC STATUS OF NATURAL POPULATIONS OF *Octopus vulgaris* USING GENOMIC TOOLS: A CANDIDATE SPECIES FOR SUSTAINABLE AQUACULTURE

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## Introduction

The common octopus (*Octopus vulgaris*) is an ecologically and economically valuable cephalopod distributed across the eastern Atlantic and Mediterranean Sea. Due to its high commercial demand, fisheries targeting this species are intense, raising concerns about the erosion of genetic diversity and the long-term viability of populations. A clear understanding of genetic structure, variability, and effective population size is essential for designing conservation measures and sustainable management strategies capable of preserving adaptive potential under growing anthropogenic pressures.

## Materials and Methods

Samples of 117 individuals were collected from seven Atlantic sites and one Mediterranean location. Restriction-site associated DNA sequencing (RAD-seq) produced genome-wide single nucleotide polymorphisms (SNPs), with 15,548 high-quality markers retained after filtering. Population structure was inferred using discriminant analysis of principal components (DAPC) and ADMIXTURE modelling. Genetic differentiation was quantified with pairwise fixation indices ($F_{ST}$), and isolation-by-distance was tested using a Mantel test. Inbreeding coefficients were estimated, and contemporary effective population sizes ($N_e$) were calculated based on genome-wide linkage disequilibrium.

## Results

Population structure analyses revealed a pronounced latitudinal genetic gradient across the Atlantic distribution of common octopus. ADMIXTURE modelling identified two ancestral clusters (K = 2), corresponding to European and African populations (Figure 1), with the Mediterranean sample exhibiting a partially distinct genetic signature at higher resolution. PCA and DAPC supported this structure, showing a clear north-south separation and highlighting limited differentiation among northern European populations. Pairwise fixation indices ($F_{ST}$) ranged from 0.000 to 0.270, with a mean of 0.116, indicating moderate genetic differentiation overall. The Mantel test confirmed a significant isolation-by-distance pattern ($r = 0.857$, $p < 0.001$), suggesting restricted gene flow driven by geographic separation. Inbreeding coefficients were elevated in several populations, particularly Mauritania, where levels of homozygosity were highest. Estimates of contemporary $N_e$ were critically low, ranging from 8 individuals in Mauritania to less than 200 across all other sites (Figure 2).

## Discussion

The pronounced latitudinal cline and moderate differentiation suggest that neutral processes, such as genetic drift and limited dispersal, shape the population structure of common octopus. The partial differentiation of the Mediterranean sample likely reflects a historical phylogeographic barrier imposed by the Strait of Gibraltar. Critically low $N_e$ values across all populations raise concerns about the long-term adaptive potential of this species. According to the widely cited 50/500 conservation rule, populations with $N_e < 50$ are at risk of short-term inbreeding depression, while $N_e < 500$ limits adaptive capacity over evolutionary timescales. In Mauritania and Morocco, where $N_e$ estimates were below 15, intensive fishing pressure combined with reduced gene flow may exacerbate the risk of genetic erosion and fitness loss. These findings highlight the urgent need for region-specific fishery management plans that integrate genomic indicators, alongside traditional demographic and ecological data. Continuous genetic monitoring will be essential to mitigate overexploitation under changing environmental pressures. Furthermore, the implementation of controlled aquaculture programs for *O. vulgaris* could help alleviate fishing pressure on natural populations, safeguard wild stocks, and promote a sustainable and economically viable production system.

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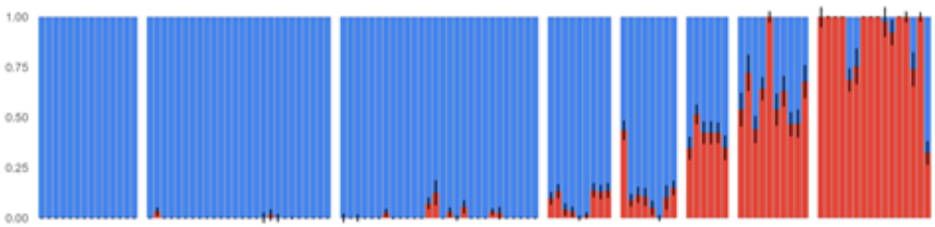


**Figure 3.** ADMIXTURE plots for $K = 2$ (optimal). Each bar represents an individual, and each colour indicates the proportion of an ancestry component. Error bars (± standard error, calculated with 200 bootstrap replicates) are shown.

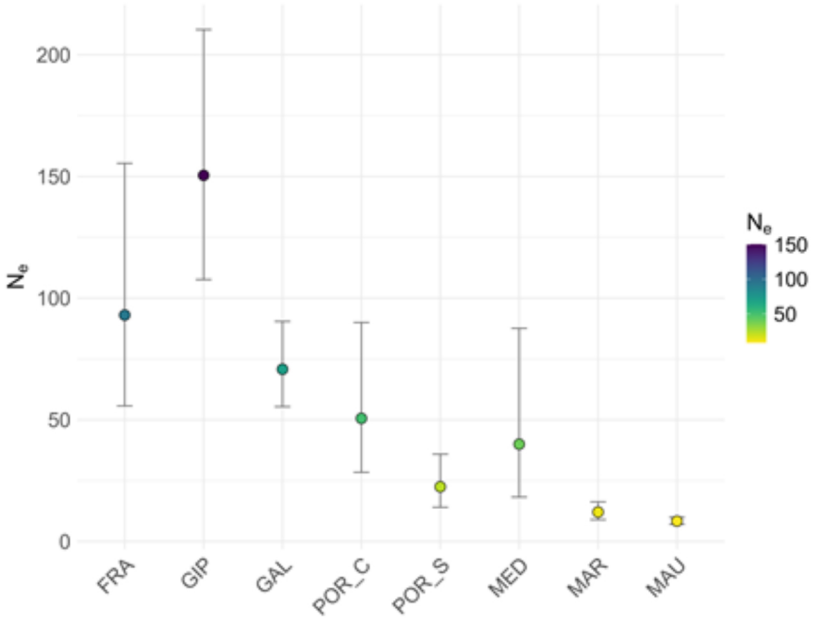


**Figure 6.** Effective population size estimations with a 90% confidence interval, obtained with CurrentNe2.

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# EXPRESSION LEVELS OF GENES ASSOCIATED WITH GAMETOGENESIS IN RAINBOW TROUT (*Oncorhynchus mykıss*) FED WITH PROBIOTIC ADDITIVE DIET

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## Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most preferred species in aquaculture due to its high commercial value and widespread use as a food source for human consumption. Owing to its economic and biological significance in aquaculture, various studies have been conducted to ensure the sustainability of this species. Among these efforts, the use of probiotic-additive (PBa) feeds has gained considerable attention. For this purpose, Lactic acid bacteria have been identified as the most frequently used probiotics in this field (Torres-Maravilla et al., 2024). Most studies on PBa diets have focused on maintaining the physiological health of fish and enhancing their immune systems (Mondal et al., 2022). In addition, probiotics in aquaculture have been reported to improve growth (Carnevali et al., 2017) and reproductive performance (Akbari Nargesi and Falahatkat, 2023), increase the expression levels of genes involved in gametogenesis and gamete quality (Safari et al., 2022;). In these studies, rainbow trout, other species such as ornamental fish, Nile tilapia (*Oreochromis niloticus*), zebrafish (*Danio rerio*), and African catfish (*Clarias gariepinus*) were used. In the present study, rainbow trout were fed with diets supplemented with the probiotic strains *Carnobacterium maltaromaticum* and *Lactococcus lactis*. At the beginning and end of the feeding period, tissue samples (liver, testis, ovary, brain, and pituitary) will be collected to analyze the expression profiles of steroidogenic genes associated with the gametogenesis process, aiming to evaluate the effects of the probiotic- additive diets.

## Material and Methods

The aim of this study was to determine the effects of Probiotic Additive (PBa) diets on the expression levels of steroidogenic genes involved in the gametogenesis process in rainbow trout (*Oncorhynchus mykiss*). The experimental procedures were conducted at the Sapanca Inland Fisheries Research and Application Unit of the Faculty of Aquatic Sciences, Istanbul University. During the experimental period, a total of 90 broodstock fish were used, with each of the six tanks containing 7 female (♀) and 8 male (♂) individuals. Probiotic Bacteria in freeze-dried form produced within the scope of the TÜBİTAK-UPAG supported project were added to commercial rainbow trout feed. The probiotic bacteria used in the study were *Carnobacterium maltaromaticum* and *Lactococcus lactis*. A total of six experimental groups were established; two control groups (negative and positive controls) and four treatment groups receiving either *C. maltaromaticum* and *L. lactis* at two different final concentrations in the feed ($10^7$ and $10^9$ CFU $g^{-1}$). Sunflower oil was used as a carrier in the preparation of the feeds for both the positive control and probiotic treatment groups. In order to investigate the effect of PBa diet on gametogenesis in rainbow trout, PBa diet was applied to individuals two months before the reproduction time.Gene expression analyses were performed at two time points; at the beginning (Day 0) and at the end of the experiment. During the feeding trial, tissue samples were collected from a total of 36 broodstock fish, consisting of 5 females (♀) and 5 males (♂) from each experimental group. Brain, liver, pituitary, testes, and ovary tissues were dissected from rainbow trout and kept in RNAlater® solution and stored at -86°C and after that RNA isolation was carried out. Following RNA isolation, complementary DNA (cDNA) synthesis will be performed. The isolated RNA will be expression levels of steroidogenic genes using real-time polymerase chain reaction (qRT-PCR) methodology. The steroidogenic genes analyzed in the present study included; *lh* (pituitary) (Hou et al., 2016), *cyp19b1* (female, brain) (Chen et al., 2021), *star* (ovary) (Bhat et al., 2024), *star* (testis) (Kusakabe et al., 2006), *erβ1* (female, liver) (Chen et al., 2021), *vtg* (ovary and liver) (Chen et al., 2021), *hsd3b1* (gonad) (Vizziano et al., 2007), *dmrt1* (testis) (Vizziano et al., 2007), *amh* (testis) (Vizziano et al., 2007), *β-actin* (all tissues).

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# EFFECT OF PROBIOTICS ON RAINBOW TROUT (*Oncorhynchus mykiss*, WALBAUM, 1792) GAMETE QUALITY

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## Introduction

Ensuring the sustainability of cultured species depends on gamete quality, and the most important factor affecting it is the composition of the feed. Probiotics have been characterized as live microorganisms that colonize the host's gut and provide health benefits when added to feeds. The benefits of probiotics are bactericidal activities and immune activating properties. Although there is a relationship between the microorganism population in the fish gut and fertilization (Gioacchini et al. 2014), the information on probiotic applications is limited. Based on the limited information, it has been determined that probiotics added to the diet improve reproductive performance, as well as gamete quality in zebrafish (Gioacchini et al. 2014; Safari et al. 2022), rainbow trout (Akbari-Nargesi et al., 2019), killifish (Lombardo et al., 2011) and Nile tilapia (Abdelal et al. 2021).

## Materials and methods

Experimental trials were conducted at the Sapanca Inland Fisheries Research and Application Unit of the Faculty of Aquatic Sciences, Istanbul University. In the study, *Hafnia alvei* + *Carnobacterium maltaromaticum* (P4) and *Lactococcus lactis* (P9), which were isolated within the scope of the TÜBİTAK project number 222N019, produced and lyophilized by MCS Aquatic R&D Aquaculture Inc. Company, were used as probiotic bacteria. Experimental groups were established, including a control group and four treatment groups supplemented with either P4 or P9 at two different final concentrations in the feed ($10^7$ and $10^9$ CFU $kg^{-1}$). Sunflower oil was added to the feed of experimental groups. Gamete quality parameters measured for both male and female trout included total fecundity and egg diameter for females, and total motility, motility duration, sperm volume, VCL (curvilinear velocity), osmolality, and pH for males. Additionally, fertilization and hatching rates were calculated for both sexes. Statistical analyses were calculated using Statistica software.

## Results

Fecundity and egg diameter, as indicators of gamete quality in females, did not show any significant differences between treatments ($P > 0.01$). Similarly, for males, parameters including total motility, motility duration, sperm volume, curvilinear velocity (VCL), osmolality, and pH did not show significant differences across the treatment groups ($P > 0.01$). However, the groups treated with P4 at $10^7$ and $10^9$ CFU $kg^{-1}$ showed a better performance in fertilization rates using female individuals from the treated groups and males from the control group ($P < 0.01$), while hatching rate from the same fertilization groups did not show significant differences ($P > 0.01$). Contrary, male individuals used from the treated groups and fertilized with females from the control groups were not significantly different across treatments ($P > 0.01$).

**Discussion and conclusions**

The aim of this study was to determine the effects of probiotic additive diets on gamete quality in rainbow trout (*Oncorhynchus mykiss*). Studies such as those by Akbari et. al., (2019) in rainbow trout and Gioacchini et al. (2014) in zebrafish reported an increase in total fecundity, as well as in the number and diameter of eggs produced by females treated with probiotics, compared to the control group. This contrasts with the results obtained in the present study, where no significant differences were observed between the groups. In the group where females were treated with probiotics and fertilized with males from the control group, a higher fertilization rate was observed; however, the hatching rate was not affected. These results are consistent with those reported by Lombardo et al. (2011) in killifish, but not with those of Gioacchini et al. (2014), in which hatching rates were affected by probiotic treatment. On the other hand, an increase in fertilization and hatching rates in rainbow trout following probiotic supplementation has also been reported by Akbari et. al., (2019).

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# PROGRESS OF AQUACULTURE ATTEMPS ON THE COLLAGEN-RICH SPONGE *Chondrosia reniformis* – IMTA APPROACH

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## Introduction

The global market demand for collagen is increasing (CAGR 6.52% until 2027). Due to its special properties such as high biocompatibility, good bioactivity, and weak antigenicity, collagen is a protein with a wide range of applications. It is used in pharmaceuticals, biomedical products, as well as in the nutritional supplements, cosmetics, and food sectors. Although terrestrial sources of collagen are numerous, outbreaks of various diseases in terrestrial animals complicate its use in humans. Marine collagen represents a promising alternative. Marine sponges are a highly valuable source of marine collagen. Among them, *Chondrosia reniformis* is of particular interest due to the high concentration (> 30%) of type I collagen in the sponge biomass (Pozzolini et al. 2012; Swatschek et al., 2002) and the absence of any inorganic components (spicules).

A major constraint regarding the industrial application of sponge collagen is the sufficient supply of sponge biomass. Promising efforts have been made in the mariculture of *C reniformis* targeting sustainable production of sponge biomass needed for collagen production (Gökalp et al, 2020; Orel et al, 2021), but the success of sponge farming is influenced by naturally fluctuating conditions such as light, current, and turbidity (Gökalp et al., 2020; Wilkinson and Vacelet, 1979). Predation, disease, and fouling can also harm this species in mariculture. Reports of sponge diseases have increased in recent decades, leading to depletion of sponge populations throughout the Mediterranean (Webster, 2007). In closed, recirculating aquaculture systems, optimal species-specific cultivation conditions can be established, enabling sustainable, reliable, and location-independent production of sponge biomass and, as a result, maximized collagen production.

The concept of integrated multitrophic aquaculture (IMTA) represents an even more sustainable approach. IMTA is based on the ecological concept of efficient reuse of organic and inorganic sidestreams through the application of different trophic levels of the food web within the culture system. Pronzato et al. (1998) were the first to include sponges in IMTA. Sponges can remove large amounts of organic matter from large volumes of water through very rapid and efficient filtration of a wide variety of suspended particles (Weisz et al. 2008; Riisgård et al. 1993; Reiswig 1974).

The efficient filtration of organic particles by sponges, coupled with their ability to produce commercially interesting natural compounds (Gökalp et al. 2019; Müller et al. 2009), is driving the interest in integrating these animals into IMTA approaches. However, examples of commercial applications of sponge farming are still rare (Duckworth 2009), and the inclusion of sponges as an element in IMTA is still in its infancy.

The focus of this study is the joint cultivation of the sponge species *C. reniformis* together with commercially relevant macroalgae and fish in an IMTA approach.

## Materials and methods

The experimental phase will start in July 2025.

***Chondrosia reniformis:*** The sponge specimen are obtained by diving from the Adriatic Sea, transported to the recirculation aquaculture system at the Alfred Wegener Institute, Bremerhaven, Germany and kept for an acclimatization period of two weeks in culturing tanks with artificial seawater. They are fragmented for growth experiments, placed on clay dishes in two divers systems: a shelf system (6 x 0.05 $m^3$) and a circular tank (1 $m^3$). They are fed once daily with a self-designed powder feed (2 ml/50, project AkPhaKol), which is dissolved shortly before feeding.

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Three experiments are conducted during the project: one experiment on the growth behavior of the sponge fragments during the IMTA assembly and two additional experiments on the targeted increase of the collagen content in the IMTA assembly. 1) Increase in vitamin C intake as feed additive or as direct application. 2) Application of silicate in the form of a) sand and b) diatom fragments. During the experiments, the survival of the sponge fragments is documented at regular intervals, and growth is calculated in g and $cm^2$ at the beginning and end of each experiment. The collagen content of the sponge fragments is determined as a percentage of dry weight at the beginning and end of each experiment.

***Ulva lactuca:*** Free-floating algae are obtained from macroalgae project (Novafoodies) conducting at the AWI. Those are distributed in four cascaded tanks (each 0.06 $m^3$). After determining the ammonium, nitrate, and phosphate levels, the cascade system is connected to the sponge cycle. The nutrient concentrations (ammonium, nitrite, nitrate, phosphate) in the sponge and algae tanks are regularly monitored. If the algae are undersupplied with nutrients, the connection to the sponge tank can be removed if necessary to ensure additional food supply. The growth and photosynthetic efficiency parameters of the algae in the cascade-system are measured every three weeks.

***Dicentrarchus labrax*:** Juveniles of European sea bass are obtained (approximately 5g body weight) from a commercial European fish farm. After an acclimation period of one week and removal of group 0 for measurement of length, weight, and health status, the fish tank is connected to the sponge-algae system. To ensure consistent conditions for the IMTA system, the following conditions are continuously evaluated: Stocking size and density, feed quantity and size, documentation of health status and feed availability for the sponge tank: The amount of feed available in the form of feces and fish food residues is documented. Additionally, water quality (ammonia, nitrate, nitrite, and phosphate levels) is monitored.

**Expected Outcome**

The location-independent, constant, and controllable conditions in a closed aquaculture system represent an optimal starting point for the development of refined sponge biomass and collagen. In former projects, an ex-situ aquaculture system for the sponge species *C. reniformis* has already been designed, constructed and adapted, in addition a feed specially tailored to the needs of the sponge *C. reniformis* has been developed. The collagen content in the obtained sponge fragments increased depending on the amount of this feed, resulting in a higher percentage of collagen to be extracted per sponge fragment.

The aim of the joint cultivation of macroalgae and fish in IMTA is to improve the maintenance and growth conditions for the sponge species *C. reniformis*. The macroalgae improve water quality by filtering nutrients such as ammonium and nitrate from the culture water and using them as food. Furthermore, the feces and food residues in the fish tanks increase the proportion of dissolved and particulate carbon in the process water, which serves as additional and constantly available food for the sponges in addition to the daily feed. Further, collagens with special properties can be produced and *C. reniformis* can be used as a sustainable collagen source on an economically relevant scale.

The concept of integrated multi-trophic aquaculture closes nutrient cycles by providing fish culture with food particles for the sponges, and using algae culture to absorb the increased nitrogen compounds in the culturing water. In an integrated multi-trophic aquaculture system, in which sponges are cultivated together with other economically relevant species, species-specific water quality and high fitness and growth of the sponge fragments are expected. In addition to the use of residual streams from fish culture, the integration of co-cultivated organisms leads to more diverse products with commercial value than monoculture

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# EFFECTS OF DIETARY VITAMIN C ON THE GROWTH AND COLLAGEN LEVEL OF THE MARINE SPONGE *Chondrosia reniformis*

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**Background**
Collagen plays an important role in healthcare and overall well-being, with numerous potential applications in areas such as drug delivery, cosmetics and the food industry. As a result, the global collagen market is expanding rapidly. Marine sponges are considered a valuable source of marine collagen, with *Chondrosia reniformis* being of special interest. This species contains a high concentration (over 30% of DW) of type I collagen in its biomass (Ghosh et al., 2019) and lacks inorganic structural components like spicules. In *C. reniformis*, ascorbic acid plays an important role as a reducing agent in the biosynthesis of collagen (Cerrano et al., 1999). During collagen synthesis, ascorbic acid is essential for the hydroxylation of proline and lysine residues, stabilizing the structure of collagen.

A major constraint regarding the industrial application of sponge collagen is the sufficient supply of sponge biomass. Land-based aquaculture represents an interesting economic approach to grow marine sponges under species-specific conditions and, as a result, maximized collagen production.

**Materials and methods**
*Chondrosia reniformis* were obtained from the sampling area in the Mediterranean Sea near Ancona/Italy and subsequently reared in a recirculation aquaculture system at the Centre for Aquaculture Research, Bremerhaven/Germany. Sponge specimens were cut and sponge fragments were allowed to attach to different substrates (oyster shells and clay plates). Sponge fragments were fed twice a day with a self-designed liquid feed with two different vitamin C concentrations (2.5 mg/ml and 22.5 mg/ml). Attachment, fragments growth and survival rate were measured at the end of the experiment. Sponge collagen was isolated (Swatschek et al, 2002) and collagen content was determined before and at the end of the experiment.

**Results**
Attachment and survival rate during the experiment were high with 86% and 100%, respectively. Collagen content of *C. reniformis* was not significantly affected by either the dietary vitamin C concentration or the substrate they grew on. *Chondrosia* attached to clay plates had a mean collagen content of 30% when fed with a vitamin C concentration of 22.5mg/ml. Cultivation on oyster shells resulted in mean collagen contents of 27%. *Chondrosia* growing on oyster shells and a vitamin C concentration of 2.5 mg/ml showed a collagen content of 26% and 27% when attached to clay plates. However, there was a slight increase in the collagen content from the beginning of the experiment to the end.

The dietary vitamin C concentration and substrate had no significant effect on the growth of *Chondrosia*. Overall, a negative growth was detected under all experimental conditions.

**Conclusion**
Land-based aquaculture of *C. reniformis* is a promising approach to grow marine sponges and increase their collagen content. Our study revealed that attachment and survival rates of *C. reniformis* during the experiment were high, at 86% and 100% respectively, indicating good viability. The collagen content was not significantly influenced by dietary vitamin C concentration. A slight increase in collagen content was observed from the start to the end of the experiment, but overall, there was no significant effect on growth, as all conditions showed negative growth. These findings suggest that while *C. reniformis* maintains collagen production independent of vitamin C supplementation, its growth may be limited under the experimental settings. This work highlights the need for further research on optimizing growth conditions for aquaculture or biomedical applications.

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# TRANSFORMING AFRICAN CITY REGION FOOD SYSTEMS THROUGH CIRCULAR AGRI-FOOD TECHNOLOGIES: THE INCITIS-FOOD PROJECT

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The EU-funded project INtegrated and Circular Technologies for Sustainable city region FOOD systems in Africa (INCiTiS-FOOD) addresses the urgent need to improve food and nutrition security (FNS) in African urban areas while reducing environmental impacts and fostering circularity. Operating across six countries, Kenya, Ghana, Nigeria, Sierra Leone, Cameroon, and Gabon, eight Living Labs serve as innovation hubs where local actors co-design, test, and adapt agri-food technologies to context-specific needs.

Core technologies include soilless cultivation systems (hydroponics, aquaponics, bioponics), recirculating aquaculture systems, and insect and snail farming. These solutions are designed to require minimal land, water, and financial inputs, making them particularly suitable for urban and peri-urban settings. The Living Labs follow an iterative design process, developing and optimizing prototypes into minimum viable products (MVPs) that meet local technical, societal, and business requirements.

In the first half of the four-year project, all Living Labs became operational, established prototype systems, and initiated multi-actor co-creation processes. Cross-Lab networking and knowledge sharing have fostered capacity building, while early results demonstrate resilience to challenges such as political instability, climate extremes, and resource constraints. The next phases will focus on scale-up, entrepreneurship incubation, and generating evidence to guide urban food policy in African cities.

By integrating technological innovation with inclusive stakeholder engagement, INCiTiS-FOOD aims to deliver scalable circular agri-food solutions, strengthen local food value chains, and contribute to sustainable, resilient city-region food systems.

# JOURNEY OF A CORAL: SCALING REEF RESTORATION THROUGH AI-DRIVEN DIGITAL TWINS

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The Shushah Island Reef restoration project, located in the Northern Red Sea off the coast of NEOM, is the world's largest active coral restoration initiative—aiming to restore 100 hectares of reef by outplanting 2 million corals across 300 native species. This presentation outlines the full lifecycle of coral propagation—from wild colony sourcing and nursery production to outplanting and long-term reef monitoring—within a digitally managed, AI-enhanced framework.

Central to this approach is the *eCoral™ Digital Twin*, an orchestration platform integrating real-time sensor data, image analytics, environmental logs, and predictive models to optimize nursery management, coral health tracking, and restoration success. AI models assist in identifying ideal genotypes for site-specific deployment, simulate environmental stressors, and forecast spawning and survivability rates—enabling adaptive reef management at industrial scale. Data from ex-situ (land-based) and in-situ (reef-based) nurseries feed into coral growth simulations and digital reef mapping to enhance restoration planning.

The integration of AI, digital twins, and ecological science offers a replicable blueprint for scaling coral reef restoration globally, while supporting biodiversity recovery, blue carbon valuation, and resilience against climate change. This talk will share lessons from operationalizing coral production at scale and the transformative role of technology in nature-based solutions.

# ACIDIFICATION CHALLENGE AFFECT THE GILTHEAD SEA BREAM SKIN MUCOSA

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## Introduction

Global climate change and anthropogenic pressures are exerting profound effects on marine fish populations and aquaculture systems. Among current environmental threats, ocean acidification represents a major concern. Driven by ongoing atmospheric $CO_2$ emissions, this phenomenon can negatively impact all marine life by limiting both growth and survival. Temperate fish species are highly sensitive to environmental fluctuations, including elevated temperatures, water acidification, and heavy metal contamination, all of which can induce physiological stress. These stressors primarily affect external tissues, such as the gills and the skin, which serve as the primary barrier between the internal and external environment, often triggering structural changes like epithelial cell damage, mucous cell hypertrophy and hyperplasia, chloride cell proliferation, and pigment cell migration within the skin (Kalogianni *et al.,* 2011). It is known that the mucosal barriers of fish undergo significant structural and molecular changes in response to altered pH, including epithelial hypertrophy, tight junctions modification, and antioxidant gene regulation (Mohammadi et al., 2019). Goblet cells, the principal mucus-secreting cells of the epidermis, are integral to this protective function. Little is known about how chronic exposure to lowered pH influences epidermal architecture and mucosal response in gilthead sea bream. This study aims to evaluate the effect of a controlled acidification challenge (pH 7.9 *vs.* pH 7.3) on sea bream alevins, integrating histological and non-invasive analyses of key skin mucus bioindicators (Sanahuja *et al.,* 2023). This approach may also contribute to the development of robust, non-invasive tools for health monitoring in aquaculture under changing environmental conditions.

## Material and methods

Gilthead sea bream alevins were exposed to two different pH conditions (7.3 and 7.9) for a period of 10 weeks under controlled laboratory conditions. Each pH treatment was maintained in triplicate tanks, and a total of 12 fish per condition were sampled at the end of the experimental period. The skin mucus was obtained and analised as described in Fernandez-Alacid *et al.,* (2018). The histological analysis was performed by fixing the skin in Bouin's solution for 24 h at room temperature, dehydrated in graded ethanol, cleared in xylene, embedded in paraffin, and sectioned at 3 μm. After dewaxing and rehydration, sections were mounted on glass slides and stained with periodic acid-Schiff (PAS) and Alcian blue (AB). Slides were digitized at 600 dpi and analyzed using ImageJ program. Epidermal thickness and mucous cell parameters were measured from randomly selected fields per sample. The skin barrier status was assessed as described in Dang *et al.* (2020) using 3 different indices:

a) Mean mucous cell (MC) area (μm2)
b) Mean MC volumetric density (%) = (MC area MC number)/(Epithelial area) x 100
c) Barrier status = 1/(MC area MC density) x 100

## Results and discussion

Exposure to pH 7.3 led to notable changes in mucus secretion and composition. Under acidified conditions, a reduction in exuded mucus volume and soluble fraction was observed, suggesting an increase in structural components. Stress-related metabolites (lactate and cortisol) were elevated in fish exposed to pH 7.3, while antioxidant power showed an upward trend, but not significant, potentially enhancing mucosal defensive sensitivity. Although mean goblet cell area was not significantly different between groups (pH 7.3: 83.8 ± 33.6 μm²; pH 7.9: 77.6 ± 17.2 μm²), histological assessment revealed a higher proportion of small-sized goblet cells (0–50 μm²) in the acidified group. This indicates increased mucus cell turnover, supporting the observed boost in secretion. No significant differences were found in goblet cell volumetric density (pH 7.3: 3.66 ± 2.52%; pH 7.9: 4.06 ± 2.28%) or in the calculated barrier index, suggesting that barrier integrity remained intact despite cellular changes. These results will be subjected to further analysis within the multidisciplinary framework of the ACUISOST project, which aims to determine how new feed formulations can interact in productive terms with the acidification of seawater.

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**Conclusions**

The preliminary results indicate that ocean acidification results in a reduction in skin mucus exudation, with a lower soluble fraction and a less optimal composition, accompanied by increased levels of stress-related metabolites. These findings suggest that long-term exposure to moderate acidification (pH 7.3) alters the skin mucosa of gilthead seabream mainly by modulating mucus cell dynamics, while preserving epidermal structural integrity. Despite the reduction in mucus exudation, no significant changes were observed in goblet cell area, volumetric density, or barrier index. Notably, the acidified group showed a higher frequency of small-sized goblet cells (0–50 μm²), indicating an increased mucus cell turnover as a compensatory mechanism to maintain mucosal defense.

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# THE MEDITERRANEAN SCALLOP *Pecten jacobaeus* L. SPAT COLLECTION AND GROWTH IN THE KRKA RIVER ESTUARY (CROATIA)

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## Introduction

Mariculture is the main source of bivalves globally, but this supply is often limited to a few species. In the Mediterranean Sea, the primary bivalves being farmed are mussels and oysters, which highlights the need to introduce new species. The Mediterranean scallop (*Pecten jacobaeus* Linnaeus 1758) is a commercially sought-after species mostly targeted by beam trawlers in the northern Adriatic Sea. However, constant fishing pressure has led to decreased landings in recent years. *Pecten jacobaeus* has a high market value and, according to previous research, can reach market size in just 18 months, with high survival rates, making it a potential candidate for aquaculture. The biggest challenge in farming this species, however, is the lack of a reliable and inexpensive source of seed.

The aim of this study was to investigate the potential for collecting juvenile *P. jacobaeus* from nature - referred to as wild spat collection - and to monitor their growth in farming installations, specifically lantern nets, for future aquaculture purposes.

## Materials and methods

Collectors, which were mesh bags filled with netron filament nets, were deployed each month from a raft at a bivalve farm located in the Krka River estuary, between the fall of 2024 and the summer of 2025. Two types of collectors, differing in mesh size (10 mm and 1 mm) were placed at various depths, ranging from 8 to 20 meters in 2-meter increments. After two months of submersion, the collectors were retrieved and examined for collected juveniles, which were then counted based on collector type and depth. Collected juveniles were measured and then placed in lantern nets at two depths, 8 and 15 m. Each subsequent month juveniles were measured again to estimate their growth. During the experiment environmental parameters, such as temperature, salinity and chlorophyll a, were monitored.

## Results

Our results indicate that the collection of *P. jacobaeus* juveniles from the Krka River estuary was highest during the spring and fall seasons. We observed that collectors with larger mesh sizes captured more juveniles than those with smaller mesh sizes. Additionally, collectors that were positioned at greater depths yielded the highest numbers of collected juveniles, with a decline in numbers closer to the surface.

The growth rate of juveniles was fastest during the spring and fall seasons. We found that juveniles placed in lantern nets at lower depth exhibited better growth compared to those placed in lanterns at greater depth, although growth varied among individual juveniles. The survival rate was low overall, with the highest mortality recorded among the smallest juveniles, which decreased as the juveniles grew larger.

## Discussion

The collection of juvenile *P. jacobaeus* from the Krka River estuary coincide with the scallop's reproductive cycle, as larvae attach to collectors 3-4 weeks after spawning. The highest numbers of juveniles were recorded following the spring and fall spawning periods. Collectors with larger mesh size yielded better results, likely because larvae could more easily enter these collectors, while smaller mesh sized collectors became clogged with fouling. The increased collection of juveniles at greater depths is attributed to the fact that *P. jacobaeus* is a benthic species; therefore, the highest numbers of larvae are found closer to the bottom.

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The faster growth of juveniles during spring and fall can be linked to the optimal temperature conditions and the abundance of available food, which peak during these seasons. Greater mortality rates were observed among the smallest juveniles, as they are most vulnerable to changes in conditions and handling. In contrast, the mortality rate decreased as juveniles grew larger and became more resilient.

**Conclusions**

Collecting *P. jacobaeus* spat from the wild is most effective during the spring and fall seasons after spawning occur. Using collectors with larger mesh sizes (approximately 10 mm) and placing them at greater depths results in better juvenile collection. Juvenile growth is rapid but depends on ambient temperature and food availability, and there are high mortality rates during the early stages of development. The number of juveniles typically collected is insufficient for starting commercial aquaculture production, so alternative methods for acquiring juveniles are necessary.

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# SUPPLEMENTATION WITH NUCLEOTIDES COMBINED WITH SEAWEED MEAL IMPROVES SURVIVAL UPON AHPND CHALLENGE IN PACIFIC WHITE SHRIMP

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Acute hepatopancreatic necrosis disease (AHPND) is a bacterial disease mainly caused by *Vibrio parahaemolyticus* which causes high mortality rates in Pacific white shrimp (PWS), *Litopenaeus vannamei*, and consequently leads to substantial economic losses. Dietary nucleotides modulate the immune response and have been shown to improve health of PWS, and seaweed products contain bioactive compounds with reported benefits in several animal species. The study was aimed at evaluating the effects of combining nucleotides (NU; Nucleoforce®, Bioiberica S.A.U., Spain) and a *Macrocystis integrifolia* seaweed meal (SM; Algaemix®, Algaex, Peru) on PWS survival upon challenge with an AHPND-causing *V. parahaemolyticus* strain.

After acclimatation for 2 days, 720 PWS (0.57±0.11 g) were classified into six groups (4 replicates/group; 30 PWS/90L-tank) and received different diet supplementation for 28 days: one group with no challenge (NC, Negative Control) and five groups challenged by immersion with *V. parahaemolyticus* and supplemented with either 500ppm NU (NU500), 500ppm SM (SM500), 500ppm NU with 500ppm SM (NU500SM500), 334ppm NU with 666ppm SM (NU334SM666) or no supplementation (PC, Positive Control). PWS were followed for 10 days post-challenge.

After 10 days, the PC group showed a significantly lower survival rate (30.63±4.70%) vs NC (88.29±2.73%; $p<0.05$). A non-significantly increased survival rate was seen with NU500 (33.95±9.62%), SM500 (41.02 ± 3.17%) and NU500SM500 (45.00±13.54%), while NU334SM666 achieved a significantly higher survival rate (59.61 ± 9.55%; $p<0.05$) (Figure 1).

In conclusion, the 1:2 combination of nucleotides with SM has a synergistic effect, reducing mortality in AHPND-challenged PWS. This innovative and sustainable nutritional intervention could therefore be used to improve PWS health, especially as an alternative to conventional management approaches to AHPND which often have limited success and negative consequences, such as antibiotics and disinfectants.

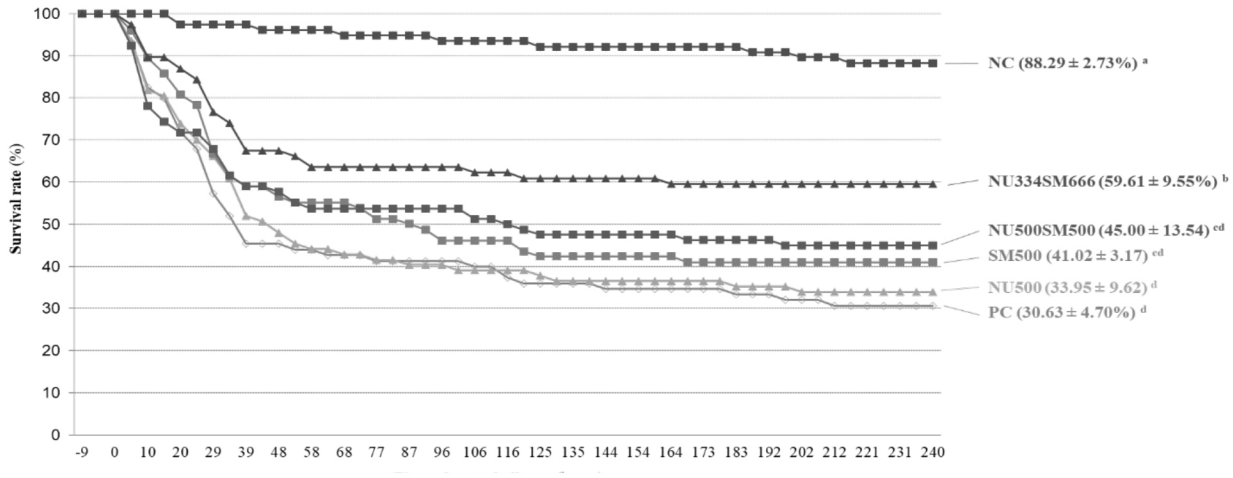


Figure 1. Survival rate of PWS after AHPND challenge. Different superindex letters indicate statistically significant differences ($p<0.05$).

# UNLOCKING EU-APPROVED HEALTH AND NUTRITIONAL CLAIMS FOR CONSUMERS OF *Sparus aurata* FED BY ALTERNATIVE FEED

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## Introduction

As aquaculture intensifies, optimizing fish nutrition remains a critical aspect of improving growth performance, health status and product quality, all of which are closely related to diet composition and nutrient availability (Glencross et al., 2020). Fundamental to the success of this sector is the formulation of nutritionally balanced feeds, in which fatty acids play a key role in supporting fish growth, health and overall product quality. Carnivorous fish species such as gilthead seabream have dietary requirements for the essential fatty acids (EFA), especially the LC-PUFA (n–3 and n–6 series with 20 or more carbons), eicosapentaenoic acid (EPA; 20:5n–3), docosahexaenoic acid (DHA, 22:6n–3) and arachidonic acid (ARA, 20:4n–6). These fatty acids not only contribute to fish health and growth but also improve the nutritional profile of aquaculture products for human consumption, supporting cardiovascular and cognitive benefits (Calder, 2020). EPA and DHA fatty acids are essential for proper brain development, including foetus, infants and toddlers (Innis, 2007; Lauritzen et al., 2016). The European Commission authorized 9 of the 54 health claims requested for the health benefits of DHA, including visual development in children, proper brain development and proper functioning of the circulatory system (R (UE) 1924/2006). In the case of EPA, there were 82 claims requested, but only 3 were authorized, related to the proper functioning of the circulatory system. Unauthorized claims denial was based on the fact that the available scientific evidence on the claimed effects was not sufficiently substantiated (R (UE) 1924/2006).

Traditionally, fishmeal (FM) and fish oil (FO) have been the main sources of essential fatty acids in aquafeeds, considering both the biological quality of the protein, and its digestibility. However, their continued use poses significant challenges, including sustainability concerns linked to overfishing, fluctuating costs and environmental impact (Moutinho et al., 2022; Naylor et al., 2021). These problems have prompted extensive research into alternative ingredients capable of replacing FM and FO without compromising feed efficiency or the quality of the fish produced.

This study investigates the potential of alternative feed (PAP and ALT)—as substitutes for traditional fishmeal (CTRL) in the diet of *Sparus aurata* (gilthead seabream), with a focus on the nutritional and health claims authorized in the European Union. The trial assessed the fatty acid profile across two key growth stages (300 g and 800 g). Results were interpreted in the context of EU Regulation (EC) No 1924/2006 on nutrition and health claims made on foods. The findings contribute to the development of sustainable feed capable of delivering fish products with added value for human health.

## Materials and methods

Three types of feed formulations (n=3, CTRL: fish meal-based diet, PAP: processed animal protein-based diet, and ALT: alternative protein-based diet). Fish sampling took place at two growing stages: when fish reached 300 g and 800 g. The nomenclature for fish was as follows: i) individuals reached 300 g: CTRL300, PAP300 and ALT300; and, ii) individuals reached 800 g: CTRL800, PAP800 and ALT800. A Gas chromatograph coupled to a Flame Ionization Detector (GC-FID) was used for detection and semi quantification of fatty acids. Sample (50 mg) was methylated following a direct extraction-methylation method with a first basic step with sodium methoxide followed by an acid step with boron trifluoride 14% in methanol as described by Trigueros & Sendra (2015).

## Results and discussion

European Union rules on nutrition and health claims were established by Regulation (EC) No 1924/2006. This regulation is the legal framework used by food business operators to inform consumers through labelling or advertising on the beneficial effects of their products, concerning health and nutrition.

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The specification of the three authorized Health Claims (HC) other than risk reduction and children's claims are detailed as follows: i) HC 1: DHA helps maintain normal triglyceride levels in the blood. *This claim can only be used for foods that provide a daily intake of 2 g of DHA and contain DHA in combination with eicosapentaenoic acid (EPA). For a product to carry this claim, consumers must be informed that the beneficial effect is obtained with a daily intake of 2 g of DHA; ii)* HC 2: DHA and EPA contribute to maintaining normal blood pressure. *This claim can only be used for foods that provide a daily intake of 3 g of EPA and DHA. For a product to carry this claim, consumers must be informed that the beneficial effect is obtained with a daily intake of 3 g of EPA and DHA; and, iii)* HC 3: DHA and EPA contribute to maintaining normal triglyceride levels in the blood. *This claim can only be used for foods that provide a daily intake of 2 g of EPA and DHA. For a product to carry this claim, consumers must be informed that the beneficial effect is obtained with a daily intake of 2 g of EPA and DHA.* It is essential to mention that following the recommended serving size for an average consumer, a portion of fish ranges between 125-150 g. Considering the percentage of DHA and EPA in the total analysed fat for each diet in 300 g and 800 g fish, we can only apply Claim 1 to ALT800 (2.07). However, claims 2 and 3 can be used for all diets (EPA: 1.79-2.08 g per serving; DHA: 1.75-2.32 g per serving; EPA+DHA: 3.45-4.39 g per serving). If we consider a 150 g serving, all three claims can be applied to all diets for both 300 g and 800 g fish (EPA: 2.15-2.5 g per serving; DHA: 2.22-2.79 g per serving; EPA+DHA: 4.37-5.27 g per serving).

On the other hand, as to authorizing nutritional claims (NC) under European Union regulation, the established criteria are included in the following lines: i) NC1: Source of omega-3 fatty acids: *if the product contains at least 0.3 g of alpha-linolenic acid per 100 g and per 100 kcal, or at least 40 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal; ii)* NC2: High content of omega-3 fatty acids: *if the product contains at least 0.6 g of alpha-linolenic acid per 100 g and per 100 kcal, or at least 80 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal; iii)* NC3: High content of monounsaturated fats: *if at least 45% of the fatty acids present in the product come from monounsaturated fats and these monounsaturated fats provide more than 20% of the product's total energy value; iv)* NC4: High content of polyunsaturated fats: *if at least 45% of the fatty acids present in the product come from polyunsaturated fats and these polyunsaturated fats provide more than 20% of the product's total energy value; and v)* NC5: High content of unsaturated fats*: if at least 70% of the fatty acids present in the product come from unsaturated fats and these unsaturated fats provide more than 20% of the product's total energy value.* Taking the requirements into account, it can be said that both growth stage fishes of the three diets can be considered both a source and high in Omega-3 fatty acids. The CTRL300, CTRL300, and CTRL800 showed a high level of MUFA, while none of the treatments exhibit a high content of PUFA and/or SFA.

**Conclusions**

According to European Union regulations, fish raised with traditional feed is considered a healthy and nutritious option due to its beneficial levels of EPA, DHA, MUFA and omega 3 fatty acids. Furthermore, the use of the proposed alternative feeds does not compromise these properties, as the levels of these essential fatty acids remain within the recommended ranges for health benefits. This ensures that fish-fed with proposed alternative diets continue to provide the same nutritional advantages, making them a suitable and sustainable option without any detriment to their health and nutritious-promoting qualities.

# DEVELOPMENT OF SUSTAINABLE DIET FOR MALABAR GROUPER (*Epinephelus malabaricus*) USING OKINAWAN LOCAL RESOURCES

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## Introduction

Malabar grouper (*Epinephelus malabaricus*) is an economically important carnivorous fish species in Okinawa Prefecture, Japan. Aquaculture production in the region has been expanding steadily in response to rising domestic demand for high-quality marine fish. However, local farmers primarily rely on commercially available diets that may not fully meet species-specific nutritional requirements, resulting in suboptimal growth performance and feed efficiency. Furthermore, concerns regarding the environmental footprint of conventional feed ingredients and the vulnerability of supply chains have intensified interest in developing alternative feed resources. To enhance the sustainability and self-sufficiency of regional aquaculture, there is an increasing need to utilize locally available materials and promote a circular resource use approach that supports both economic viability and ecological resilience. This study aimed to develop sustainable diets for Malabar grouper by incorporating Okinawan fishmeal and livestock by-products as alternative protein sources to contribute to a regional circular resource utilization model.

## Materials and methods

Two feeding trials were conducted over 56 days in a flow-through system using 500 L tanks equipped with 45 L net baskets. Twelve fish per treatment group (initial mean weight 34.5 g) were hand-fed to near satiation. The first trial evaluated growth performance and nutrient digestibility of a diet formulated with Okinawan-produced fishmeal compared to a standard commercial fishmeal diet. The second trial assessed partial replacement of commercial fishmeal (75%) with pork-chicken meal at inclusion rates of 25%, 50%, and 75%. Apparent digestibility coefficients (ADC) of protein and lipid, body and muscle composition, and growth parameters were determined following standard analytical procedures.

## Results and discussion

The Okinawan fishmeal diet yielded slightly reduced growth (final body weight 80.1 ± 5.2 g) compared to the commercial fishmeal diet (85.4 ± 4.6 g). Protein digestibility remained high in both groups (Okinawan fishmeal: 89.3%; commercial fishmeal: 90.0%), whereas lipid digestibility was notably lower in the Okinawan fishmeal group (70.8% vs. 85.9%). In the livestock by-product replacement trial, substituting 25% of fishmeal with pork-chicken meal maintained growth performance (final weight 84.7 ± 5.1 g) and digestibility (protein ADC: 87.0%; lipid ADC: 86.9%). However, higher replacement levels (50–75%) resulted in significantly reduced protein digestibility (72.7–80.5%) and lipid digestibility (49.5–58.2%), indicating a threshold beyond which nutrient utilization declines. No adverse effects were observed on muscle proximate composition at moderate replacement levels.

These findings underline the importance of ongoing efforts to evaluate the nutritional optimization of locally sourced ingredients and to identify practical feed formulations that balance growth performance with environmental and economic sustainability. In particular, strategies to improve the lipid digestibility of Okinawan fishmeal and to refine processing techniques for livestock by-products are essential for maximizing the potential of regional resources. Future studies will also focus on conducting long-term feeding trials under commercial farming conditions to validate the efficacy of these diets and to assess their impacts on fish health and product quality.

## Conclusion

This study demonstrates that partial replacement of commercial fishmeal with locally produced livestock by-products can support satisfactory growth and nutrient utilization in Malabar grouper, highlighting the potential of Okinawan resources for sustainable aquafeed development. While further optimization of the nutritional quality of Okinawan fishmeal is required, these findings contribute to establishing a regional circular resource utilization model in line with the goals of the JST COI-NEXT project in University of the Ryukyus, which aims to promote integrated and sustainable aquaculture practices in Okinawa.

# APPLICATION OF GELATIN–CHITOSAN BASED SMART PACKAGING FILM CONTAINING ANTHOCYANINS IN FISH PACKAGING

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Smart biodegradable packaging films composed of gelatin (G) and chitosan (Cs) were developed for potential use in monitoring fish freshness. These films incorporated natural crosslinkers with antioxidant and pH-responsive properties. Anthocyanins extracted from *Echium amoenum* (EAA) were integrated as natural pH indicators, alongside citric acid (CA) and cinnamaldehyde (C) as crosslinkers. The resulting films exhibited excellent transparency and UV-blocking ability. Their color changed visibly in response to pH variation, a feature particularly useful for detecting spoilage in aquatic products. Notably, the G/Cs/CA5%/EAA film demonstrated enhanced physical and mechanical characteristics, including increased tensile strength, reduced water vapor permeability, lower elongation, and improved thermal stability, compared to the cinnamaldehyde-based film. Though the G/Cs/C5%/EAA film exhibited higher antioxidant activity (26.71%), the CA-based version showed smoother surface morphology and better overall performance. Most importantly, the films responded distinctly to chemical changes caused by fish spoilage and ammonia exposure, enabling real-time, non-invasive freshness monitoring. These findings highlight the potential of G/Cs/CA5%/EAA films as intelligent indicators in fish packaging, offering a sustainable and efficient solution for quality assurance in aquaculture and seafood distribution.

# STICK IT TO LACTOCOCCUS: DEVELOPMENT OF A POINT-OF-CARE DIAGNOSTIC ASSAY FOR PISCINE LACTOCOCCOSIS

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Piscine lactococcosis is a critical bacterial disease impacting farmed fish, predominantly caused by species within the Lactococcus genus, including *Lactococcus garvieae, Lactococcus petauri*, and *Lactococcus formosensis.* Timely and accurate diagnosis is essential for effective outbreak management and mitigation of economic losses in aquaculture. While conventional diagnostic methods provide reliable results, they often necessitate specialized laboratory infrastructure and extended processing times, which limits their practicality in field conditions.

To overcome these limitations, we have developed a novel point-of-care (POC) diagnostic assay leveraging Recombinase Aided Amplification (RAA) technology combined with a lateral flow dipstick (LFD) detection system, enabling rapid and specific identification of piscine lactococcosis causative agents. Three species-specific RAA assays were designed, each targeting distinct genetic markers of *L. garvieae, L. petauri*, and *L. formosensis.* These assays exhibited high specificity, with no cross-reactivity detected among target species or other bacterial strains tested. Sensitivity analysis demonstrated a limit of detection between 10 and 20 genome copies, underscoring the assay's capability to detect low bacterial loads, which is critical for early diagnosis. Preliminary validation using field samples from infected fish yielded promising results, with each assay accurately detecting its corresponding pathogen without cross-detection. Ongoing efforts aim to broaden field validation across diverse geographical locations and aquaculture systems to further confirm assay robustness and reliability. Additionally, assay optimization is underway to enhance usability for non-specialist personnel, including simplified sample preparation and streamlined workflow.

In conclusion, the developed RAA-LFD diagnostic assays represent a rapid, sensitive, and specific POC solution for detecting Lactococcus species responsible for piscine lactococcosis. This advancement has the potential to significantly improve disease management in aquaculture by enabling decentralized testing, reducing dependence on centralized laboratories, and supporting sustainable fish production practices.

# DOMESTICATION STRATEGY OF THE MAJESTIC HUMPHEAD WRASSE (*Cheilinus undulatus*)

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## Introduction

The humphead wrasse, also known as the Napoleon wrasse (*Cheilinus undulatus*), is a highly valuable marine fish species predominantly found in coral reef habitats across the Indo-Pacific region. Unfortunately, it is listed as Endangered on the International Union for Conservation of Nature (IUCN) Red List due to severe population declines caused by overfishing, particularly for the aquarium trade and the live reef fish food market (Sadovy & Vincent, 2002). Their slow growth rate and late sexual maturity make them highly susceptible to overexploitation, further exacerbating population declines. Given these challenges, conservation efforts are critical, including the establishment of marine protected areas (MPAs) and the implementation of sustainable fishing practices to ensure the species' long-term survival. Despite its high commercial value, aquaculture efforts for humphead wrasse remain rare, largely due to the difficulty in breeding and rearing the species in captivity. To date, only extremely rare instances of successful hatchery production of humphead wrasse seed have been documented. At the Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah (UMS), initiatives to domesticate the species began several years ago, yielding promising results in broodstock nurturing. The challenges of breeding this species stem from its complex reproductive behaviors, specific dietary requirements, and prolonged larval phase, all of which hinder large-scale aquaculture production. Additionally, its slow maturation rate presents further obstacles in achieving a sustainable breeding cycle. To advance aquaculture efforts, it is essential to establish a comprehensive baseline of biological and ecological information on this little-known species. This paper presents the ongoing strategies and early findings of humphead wrasse domestication, offering insights that could contribute to future aquaculture development and conservation management.

## Materials and Methods

A total of 65 tails of *C. undulatus* broodstocks reared in the captivity throughout six months of research period and fed daily with fresh squid (*Sepia sp.*) in a 150 ton tank at the Fish hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia. Sex determination was carried out based on morphological assessment including the presence of eyeline, hump on head and genital papillae, of the *C. undulatus* were carried out to identify the sex (Tan et al., 2019). Another cohort of fish (30 fish; 700-1200g) were subjected to morphometric and meristic characterization, as well as nutritional profile (proximate analysis, amino acids, fatty acids, vitamins and minerals) using standards methods. In the weaning experiment fish were placed in a 30 ton round tank with different feeding regimes.

## Results and Discussion

Our preliminary findings show that *C. undulatus* exhibited an isometric growth pattern with more than half of the population being under the unknown status (neither male or female). Morphologically, females were distinguished by a less pronounced hump on the head and a visible eyeline behind the eyes, which was absent in males. Additionally, the study clearly revealed that male *C. undulatus* displayed an elongated genital papilla, whereas females had a rounded genital papilla with a distinct small egg hole. Induced spawning trials using hormone treatments have been conducted, with Ovaplant demonstrating the highest potential. Only ovaries were obtained from this fish cohort. On the other hand, nutritional analysis of *C. undulatus* has been done to provide valuable insights into the nutritional profile of the fish, which can be used to develop species-specific feeds. Significant differences were observed between the dorsal and ventral fillets of humphead wrasse, highlighting distinct nutritional strengths. The dorsal portion was higher in protein, essential amino acids, and minerals such as calcium and magnesium, making it ideal for consumers seeking muscle-building nutrients and bone health support. In contrast, the ventral portion contained higher fat content, particularly omega-3 fatty acids like DHA, as well as vitamin D, offering benefits for

*(Continued on next page)*

cardiovascular health, immune function, and energy. By-products from the fish also reveal a high potential nutritional profiling with high protein and mineral contents. Meanwhile, weaning from trash fish to formulated feed took at least about 2 month with a fasting-strategy. Survival rate during weaning period remains high with a flow-through culture system in round fiberglass tanks. An on-going feeding trial is conducted to evaluate the growth performance of humphead wrasse fed formulated feeds in captivity.

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# ENHANCING SOLE LARVAL ROBUSTNESS WITH ALGAE BASED SOLUTIONS: INSIGHTS INTO GROWTH, REDOX STATUS AND GUT INTEGRITY

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**Introduction**

Marine fish larvae face significant challenges during their early development. During this period, rapid growth, and intense metabolic activity make fish larvae highly vulnerable to oxidative stress; highlighting the critical role of nutrition (Xavier et al., 2021). Algal extracts are rich in bioactive compounds, therefore understanding how these molecules modulate digestive, and redox pathways in postlarval stages of fish is essential (Aragão et al., 2022). This work explores how dietary inclusion of micro- and macroalgae may support larval robustness and resilience through a modulation of growth performance, oxidative stress response, and gut integrity in *Solea senegalensis* postlarvae.

**Materials and methods**

In this experiment, sole larvae were fed one of three experimental diets: a commercial-like diet (COM) or two experimental diets supplemented with different combinations of algal products. These combinations used products of the macroalgae *Ulva* sp. and the microalgae *Phaeodactylum* (UPD), and *Tetradesmus* sp (UTD). The experiment lasted 24 days, from 25 days after hatching (DAH) to 49 DAH (Fig.1). After 49 DAH, sole previously fed with the experimental diets (COM, UPD, UTD) were fed with a new diet (Boost) for 5 days, until the end of the experiment (54 DAH).

During the experimental period, postlarvae (initial dry weight 1.24 mg) were reared in triplicate tanks in a recirculating aquaculture system. Throughout the nutritional trial, dissolved oxygen was maintained above 99.5%, temperature at 20.7°C, and pH at 8.1. Growth performance was assessed at 49 DAH. Key oxidative stress markers – superoxide dismutase (SOD), catalase (CAT), and total glutathione (GSH) – along with indicators of macromolecule damage like lipid peroxidation (LPO), as well as the expression of genes related to antioxidant defence (e.g. sod, cat), gut integrity (e.g. *cldn15*) and immune response (e.g. *il-1b*), were assessed at 49 and 54 DAH.

**Results & Discussion**

At 49DAH, the dietary treatments had no significant effects on growth, with larvae presenting a dry weight of 31.18 mg, a total length of 25.89 mm, and a condition factor of 0.89. The relative growth rate was 14.34 % $day^{-1}$, and the survival rate was 68.41%. After feeding the larvae with the COM and algae-supplemented diets, no clear trend in the antioxidant response was observed. However, after 5 days of feeding the larvae with the boost diet (at 54 DAH), LPO was significantly higher ($p < 0.05$) in larvae fed the UTD diet compared to those fed the control (COM). A higher LPO is not a positive response. However, as reported by Xavier et al. (2021), sole larvae when fed some doses bioactive compounds can exhibited higher cellular damage, nevertheless after a stressful event may lead to improved resilience. In the present trial, since larvae were not subjected to a challenge, it was not possible to determine whether the boost diet conferred an adaptive advantage. In terms of the molecular markers related to antioxidant defence, gut integrity and immune response, larvae fed the algae-supplemented diets seemed better prepared for a potential challenge, since the expression of genes such as *sod*, *cat*, *cldn15*, and *il-1b* was upregulated compared to larvae fed the COM diet.

**Conclusion**

Supplementing microdiets with algae products rich in antioxidants and other bioactive molecules may elicit both beneficial and adverse physiological responses. This dietary fine-tuning underlines the complexity of marine fish larvae nutrition and emphasizes the importance of tailoring diets to enhance larval resilience under hatchery production.

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# SCIENCE POLICY REPORT: ADVANCING SUSTAINABILITY AND CIRCULARITY IN AQUACULTURE TO BUILD A RESILIENT GLOBAL FOOD SYSTEM

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## Introduction

Jointly organised by the Brazilian Academy of Sciences (ABC), the German National Academy of Sciences Leopoldina, and the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), the workshop "Sustainable Aquaculture – Environmental Impacts and Food Security", held in Berlin, Germany, in October 2023, brought together an interdisciplinary group of emerging scientists from Brazil and Europe to assess the potential and challenges of freshwater aquaculture in the face of rapid climate change and unprecedented loss of biodiversity. As a result of this workshop, a policy report was published by the German National Academy of Sciences Leopoldina and the Brazilian Academy of Sciences on 16 June 2025, emphasizing the prospects of especially freshwater aquaculture as a key component of a resilient, sustainable and increasingly circular global food production system, while considering the unique circumstances in Brazil and Germany and providing specific policy recommendations for both countries [1].

## Report summary

While acknowledging its many challenges, the aquaculture sector is growing rapidly and offers enormous potential to shift the global food system towards greater efficiency, sustainability and resilience. With the great variety of fish production methods, aquaculture has the ability to increase its importance as a sustainable global food source, both in terms of quantity and nutritional quality. Through the policy report, the author group aims to outline a path for aquaculture, especially freshwater aquaculture, to become a resilient, future-proof and increasingly circular mode of food production, focusing on available freshwater aquaculture technologies andoffering a comparative perspective on the fish farming sectors in Brazil and Germany.

Despite many differences, Brazil and Germany both share substantial potential for growth in the freshwater aquaculture sector. However, fish production and consumption in both countries fall below the global average, e.g., with Germany producing only 2% of domestically consumed fish. Both nations face similar challenges regarding the impacts of climate change, high production costs, negative public perception, labour shortages, and regulatory barriers. Expanding sustainable aquaculture requires strong political support, strategic measures, and efforts to enhance public awareness of its benefits compared to other animal production sectors. With the vision of expanding sustainable freshwater aquaculture, it is emphasised that research and technological advancements, as well as economic feasibility, are crucial in enhancing productivity, resource efficiency, and environmental sustainability.

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**Policy recommendations**

Aquaculture has great potential to contribute to a sustainable, circular, and resilient global food system. To unlock its ability to support healthy human diets, especially in regions with low self-sufficiency, three main objectives must be met.

1) Simplify regulatory procedures to support the sustainability of existing aquaculture enterprises and facilitate the creation of new ones.
2) Implement effective education and workforce development, while fostering consumer awareness about the sustainability and health benefits of modern aquaculture.
3) Translate scientific knowledge and innovations into practical, sustainable solutions.

To achieve these objectives, we recommend the following actions:

Policy and regulation

- Streamline bureaucracy: Limit the number of regulatory bodies responsible for licensing and controlling freshwater aquaculture facilities.
- Introduce aquaculture officers: These officers, located within regulating bodies, would guide prospective aquaculture entrepreneurs through the entire licensing and monitoring process, from initial concept to full implementation.

Consumer awareness and educating the future workforce

- Offer meals based on sustainable aquaculture products: Integrate domestically produced sustainable (freshwater) aquaculture products into public canteens, schools, and universities. This initiative can help support public health and promote the role of aquaculture in transforming the food production sector.
- Launch a mainstreaming programme: Raise societal awareness about the nutritional benefits of fish and the sustainable, secure, and transparent practices behind domestic freshwater aquaculture. Collaboration between governmental bodies, NGOs and the sector can help improve public perception and address prevailing biases against farmed fish.
- Intensify training programmes: Promote fish farming as a promising career path for young professionals. A skilled workforce is needed to scale up sustainable freshwater aquaculture, including:
    - Technical training for practitioners.
    - Academic training for research, education, and governance roles.

Science and innovation for practice

- Develop technological packages: These should be tailored to local needs and opportunities, focusing on sustainable technologies that offer economically viable solutions. These packages should include the selection of appropriate species, the re-use of by-products, and the implementation of effective waste management strategies to improve circularity.
- Co-develop local best management practices (BMPs): In collaboration with industry and government agencies, develop BMPs, guidance, and tools that encompass skills, knowledge, technology, services, infrastructure and governance. In this process, social and gender equity should also be considered.

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# REPLACEMENT EFFECTS OF FISH MEAL WITH HIGH PROTEIN DISTILLERS DRIED GRAINS WITH SOLUBLES IN THE DIET ON GROWTH PERFORMANCE AND FEED AVAILABILITY OF OLIVE FLOUNDER (*Paralichthys olivaceus*)

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## Introduction

Olive flounder (*Paralichthys olivaceus*) was the most commercially important marine finfish species in korea in 2024 with the highest production volume of 40,125 metric tons (49% of the total) and value of 684 billion KRW (57% of the total) (KOSIS, 2025). Fish meal (FM) has become an expensive protein source for fishfeeds due to decreasing production and increasing demand worldwide (Index Mundi, 2025). Thus, it is essential to find an alternative protein source for FM in aquafeeds that are year-round available and inexpensive to reach sustainable aquaculture. High protein distillers dried grains with solubles (HPD), a by-product of the corn fuel ethanol production, is a possible plant-based alternative protein source for FM. Therefore, This study aims to evaluate the replacement effects of fish meal with HPD in the diet on growth performance and feed availability of olive flounder.

## Materials and methods

Five experimental diets with isoproteic (51.0%) and isolipidic (14.0%) were formulated (Table 1). In the control (Con) diet, 60% FM was contained. In the Con diet, 10%, 20%, 30%, and 40% of FM were substituted with HPD, referred to as the HPD10, HPD20, HPD30, and HPD40 diets, respectively. A total of 600 olive flounder (6.5 ± 0.04 g; mean ± SE) were randomly distributed into 15, 300-L flow through tanks (40 fish per tank) with triplicate. All fish were hand-fed to apparent satiation twice a day for 50 days. The proximate composition of the experimental diets and whole-body fish were analyzed using the standard methods. The amino acid and fatty acid profiles of the experimental diets were analyzed following the standard methods (AOAC, 1990). Also, The plasma parameters of fish were measured. One-way ANOVA, Duncan's multiple range test, orthogonal polynomial contrast and regression analysis were conducted by SPSS version 24.0.

**Table 1.** Ingredient and chemical composition of the experimental diets (%, DM basis).

| | Experimental diets | | | | |
|---|---|---|---|---|---|
| | Con | HPD10 | HPD20 | HPD30 | HPD40 |
| Fish meal | 60.0 | 54.0 | 48.0 | 42.0 | 36.0 |
| High protein distillers dried grains with solubles | | 8.8 | 17.6 | 26.4 | 35.2 |
| Fermented soybean meal | 13.0 | 13.0 | 13.0 | 13.0 | 13.0 |
| Wheat flour | 18.0 | 15.0 | 11.9 | 8.9 | 5.8 |
| Fish oil | 4.0 | 4.2 | 4.5 | 4.7 | 5.0 |
| Soybean oil | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Choline | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Crude protein | 51.0 | 50.7 | 50.8 | 50.7 | 50.9 |
| Crude lipid | 11.8 | 11.6 | 12.0 | 11.9 | 11.8 |

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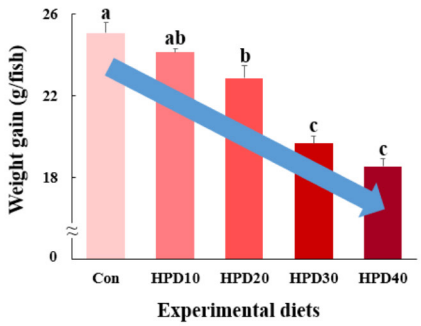


**Fig. 1.** Weight gain (g/fish) (Orthogonal polynomial contrast; **Linear = 0.0000**, Quadratic = 0.1877, Cubic = 0.1061) (**Y = – 0.174939X + 25.5451**, $R^2$ = 0.9049, $P$ < 0.0001) of olive flounder fed the experimental diets substituting increased level of FM with HPD for 50 days (means of triplicate ± SE) (***P* < 0.0001**).

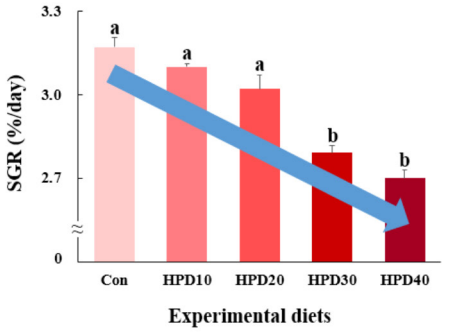


**Fig. 2.** Specific growth rate (SGR, %/day) (Orthogonal polynomial contrast; **Linear = 0.0000**, Quadratic = 0.1622, Cubic = 0.1853) (**Y = – 0.000123X + 0.0320**, $R^2$ = 0.8888, $P$ < 0.0001) of olive flounder fed the experimental diets substituting increased level of FM with HPD for 50 days (means of triplicate ± SE) (***P* < 0.0001**).

## Results

At the end of the 50-day feeding trial, Survival of olive flounder achieved over 95.8%, but it was not influenced by dietary treatments. Weight gain (WG) of olive flounder fed the Con diet was significantly ($P$ < 0.0001) higher than that of fish fed the HPD20, HPD30, and HPD40 diets, but not significantly ($P$ > 0.05) different from that of fish fed the HPD10 diet (Fig. 1). Specific growth rate (SGR) and feed consumption (FC) of olive flounder fed the Con, HPD10, and HPD20 diets were significantly ($P$ < 0.0001 and $P$ < 0.0009, respectively) higher than those of fish fed the HPD30 and HPD40 diets (Fig. 2). In orthogonal polynomial contrast, WG, SGR, and FC of olive flounder tended to linear ($P$ = 0.0001, $P$ = 0.0001, and $P$ = 0.0001, respectively) relationships with dietary increased substitution levels of FM with HPD. In regression analysis, linearly decreased relationship were determined to be the most suitable model between dietary FM substitution with HPD and WG (Y = – 0.174939X + 25.5451, $P$ < 0.0001, $R^2$ = 0.9049), SGR (Y = – 0.000123X + 0.0320, $P$ < 0.0001, $R^2$ = 0.8888), and FC (Y = – 0.126285X + 23.6996, $P$ < 0.0001, $R^2$ = 0.7421), respectively (Fig. 1 and 2, respectively). Feed utilization such as feed efficiency, protein efficiency ratio, and protein retention were not affected by dietary FM substitution with HPD. Condition factor and hepatosomatic index of olive flounder were not significantly affected by the dietary treatments. However, viscerosomatic index (VSI) of olive flounder fed the HPD30 and HPD40 diets was significantly higher than that of fish fed the Con diet. In orthogonal polynomial contrast, VSI of olive flounder tended to linear ($P$ = 0.0002) relationships with dietary increased substitution levels of FM with HPD. In regression analysis, linearly increased relationship were determined to be the most suitable model between dietary FM substitution with HPD and VSI (Y = 0.000287X + 0.064452, $P$ < 0.0002, $R^2$ = 0.6900). plasma parameters and proximate composition of whole-body fish were not affected by the dietary treatments.

## Conclusion

FM up to 10% and 20% could be substituted with HPD in the 60% FM-based diet without significantly deteriorating WG and SGR of olive flounder, respectively.

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# SUBSTITUTION EFFECT OF FISH MEAL WITH DUCK BY-PRODUCT MEAL IN THE DIET OF OLIVE FLOUNDER (*Paralichthys olivaceus*) ON THE GROWTH PERFORMANCE, FEED AVAILABILITY, AND ECONOMIC ANALYSIS

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## Introduction

Fish feeds are typically more costly than feeds for livestock, primarily due to the high inclusion of fish meal (FM), which is one of the most expensive feed ingredients. However, the FM price has increased due to reduced FM production and increased global demand (Index mundi, 2025). Therefore, various animal by-products, such as meat meal, chicken by-product meal, and tuna by-product meal have been evaluated as an alternative protein source for FM in the olive flounder diets. Since DBM has high crude protein (61.5%) and crude lipid (10.6%) content, but is less expensive (USD 0.60/kg) than FM (USD 2.16/kg) (Islam et al., 2024), it can be used as a FM replacer in olive flounder diet. This study aims to evaluate the substitution effects of FM with DBM in the diet of olive flounder on the growth performance, feed availability, and economic analysis.

## Materials and Methods

The control (Con) diet contained 60% FM and 16% fermented soybean meal as the main protein sources, 4.0% fish oil and 2.5% soybean oil as the lipid sources, and 15.0% wheat flour as the carbohydrate source (Table 1). In the con diet, 20%, 40%, 60%, 80%, and 100% FM were substituted with DBM, and referred to the DBM20, DBM40, DBM60, DBM80, and DBM100 diets. All experimental diets were formulated at isoproteic (52.0) and isolipidic (12.0%). All experimental diets were assigned to triplicate groups of fish. A total of 450 juvenile (6.3 ± 0.07 g; mean ± SE) fish were distributed into 18 of 50-L flow-through tank (25 fish/tank) with triplicate. Fish were hand-fed to apparent satiation twice a day for 50 days. After the feeding experiment, growth performance, feed availability, biological indices and, plasma parameters were evaluated. Chemical composition of the experimental diets and fish were analyzed according to AOAC methods (1990). Also, Economic conversion ratio (ECR, USD/kg) and Economic profit index (EPI, USD/fish) were measured. One-way ANOVA and Tukey's post-hoc test were applied using SPSS version 24.0. Orthogonal polynomial contrasts and regression analysis were conducted to evaluate linear, quadratic, and cubic as the best fitable model between FM substitution levels with DBM and dependent parameters, respectively.

Table 1. Ingredient and chemical composition of the experimental diets (%, DM basis).

| | Experimental diets | | | | | |
|---|---|---|---|---|---|---|
| | Con | DBM20 | DBM40 | DBM60 | DBM80 | DBM100 |
| ***Ingredients* (%, DM)** | | | | | | |
| Fish meal (FM) (CP; 69.8%, CL; 7.8%, Ash; 14.6%) | 60.0 | 48.0 | 36.0 | 24.0 | 12.0 | |
| Duck by product meal (DBM) (CP; 61.6%, CL; 10.6%, Ash; 21.2%) | | 14.0 | 28.0 | 42.0 | 56.0 | 70.0 |
| Fermented soybean meal | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 |
| Wheat flour | 15.0 | 13.5 | 12.0 | 10.5 | 9.0 | 7.5 |
| Fish oil | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Soybean oil | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | |
| Vitamin mix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral mix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Choline | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| ***Nutrients* (%, DM)** | | | | | | |
| Dry matter | 95.7 | 95.0 | 95.1 | 96.6 | 96.4 | 95.9 |
| Crude protein | 52.3 | 52.5 | 52.5 | 52.0 | 52.4 | 52.3 |
| Crude lipid | 11.9 | 11.9 | 11.5 | 11.6 | 11.7 | 11.8 |
| Ash | 10.3 | 12.4 | 13.9 | 15.5 | 16.5 | 17.0 |

*(Continued on next page)*

## Results

After 50-days feeding experiment, Weight gain (WG) and specific growth rate (SGR) of olive flounder fed the Con, and DBM20, and DBM40 diets were significantly ($P < 0.0001$ for both) higher than those of fish fed the DBM60, DBM80, and DBM 100 diets. In orthogonal polynomial contrast, WG and SGR of olive flounder showed linear ($P = 0.0001$ for both), quadratic ($P = 0.0001$ for both), and cubic ($P = 0.0001$ and $P = 0.001$, respectively) relationships with dietary substitution levels of FM with DBM. In regression analysis, cubic relationships were revealed to be the most suitable model between dietary substitution levels of FM with DBM and WG ($Y = 0.000043X^3 - 0.007713X^2 + 0.211515X + 21.5563$, $P < 0.0001$, $R^2 = 0.9566$, $Y_{max}$ = X value of 15.80%) and SGR ($Y = 0.000000033674X^3 - 0.000006X^2 + 0.000177X + 0.0299$, $P < 0.0001$, $R^2 = 0.9504$ $Y_{max}$ = X value of 17.22%), respectively. Feed consumption (FC) of olive flounder fed the Con, DBM20, and DBM40 diets were significantly ($P < 0.0001$) higher than that of fish fed the DBM60, DBM80, and DBM100 diets. In orthogonal polynomial contrast, FC of olive flounder showed linear ($P = 0.0001$), quadratic ($P = 0.0001$), and cubic ($P = 0.0001$) relationships with dietary substitution levels of FM with DBM. In regression analysis, cubic relationship was revealed to be the most suitable model between substitution levels of FM with DBM and FC ($Y = 0.000047X^3 - 0.008625X^2 + 0.275474X + 19.9047$, $P < 0.0001$, $R^2 = 0.9615$, $Y_{max}$ = X value of 18.88%). Condition factor (CF) of olive flounder fed the Con diet was significantly ($P < 0.0001$) higher than that of fish fed the DBM60, DBM80, and DBM100 diets, but not significantly ($P < 0.05$) different from that of olive flounder fed the DBM20 and DBM40 diets. However, there were no significant ($P < 0.05$) differences in feed utilization, biological indices expect for condition factor, chemical composition, and plasma parameters by dietary treatment. The highest economic conversion ratio (ECR) was observed in the Con diet ($P < 0.0001$). Economic profit index (EPI) of the Con, DBM20, and DBM40 diets was significantly ($P < 0.0001$) higher than that of the DBM60, DBM80, and DBM100 diets.

## Conclusion

DBM could substitute up to 40% of FM in the 60% FM-based diet without negatively affecting the growth performance, feed availability, and biological indices of olive flounder. The DBM40 diet maximize economic returns (EPI) for farmers.

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# SEASONAL AND SPATIAL PATTERNS IN FITNESS, REPRODUCTIVE STAGES, AND PARASITIC INFECTIONS OF THE MANILA CLAM *Ruditapes philippinarum* ALONG THE WEST COAST OF KOREA

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The Manila clam, *Ruditapes philippinarum*, inhabiting the west coast of Korea, frequently experiences infections by protozoan and metazoan parasites, adversely affecting its growth and reproductive capacity. This study investigated seasonal fluctuations in the condition index (CI), reproductive stages, and parasite prevalence in clams sampled from four tidal flats near Anmyeondo Island. Histological examinations identified four major parasites: *Perkinsus olseni* infecting gill tissues, *Cercaria tapetis* within gonads, *Parvatrema duboisi* in the mantle tissue, and echinostomatoid encysted metacercaria infecting the foot muscle. Peak spawning activity occurred from July to September, corresponding to the period of highest sea surface temperatures (SST), with minimal variation across sampling sites. Significant spatial differences in clam condition indices were noted, with higher values recorded at Bangpo (120.9) and Janggok (119.1) compared to Gonam (113.4) and notably lower indices at Shinya (84.0). The intensity of *P. olseni* infection significantly varied between sites, showing elevated counts in muddy-sand habitats (Janggok: 2.219 × 10^6 cells/g, Gonam: 1.561 × 10^6 cells/g gill tissue) relative to sandy habitats (Shinya: 0.260 × 10^6, Bangpo: 0.057 × 10^6 cells/g). Despite high *P. olseni* prevalence, clams at Janggok exhibited superior fitness, possibly benefiting from greater nutritional resources associated with sediment organic enrichment. Conversely, Shinya clams displayed the poorest fitness outcomes, likely due to suboptimal feeding conditions in sandy substrates and intense trematode infestation of foot tissues.

# MORPHOLOGICAL CHARACTERIZATION, LARVAL DEVELOPMENT, AND MITOCHONDRIAL GENOME ANALYSIS OF *Rochia* AFF. *Nilotica* (GASTROPODA: TEGULIIDAE) FROM CHUUK ATOLL, FEDERATED STATES OF MICRONESIA, WITH INSIGHTS INTO GENETIC DISTINCTIVENESS

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The button shell *Rochia nilotica* is economically valuable across Pacific Island nations, primarily due to its thick nacreous layer, widely utilized in button manufacturing and ornamental crafts. However, overfishing has led to significant population declines, necessitating detailed biological and genetic assessments to inform sustainable management practices. This study presents the first comprehensive examination of morphological characteristics, larval development, and mitochondrial genome sequencing of *R. nilotica* collected from coral reefs at a depth of 5 m near Weno Island, Chuuk Atoll, Federated States of Micronesia (FSM). Morphological analyses confirmed the characteristic features of *R. nilotica*, including an open umbilicus, distinct radial purple-red streaks, smooth columella, and variation in shell morphology with age. Eggs were approximately 50 μm in diameter, surrounded by a gelatinous layer within which fertilization and early differentiation occurred. At a water temperature of 29°C, embryos developed into veliger larvae approximately 10 hours after fertilization. Shell development and visceral torsion were observed 24 hours post-fertilization, and after 80 hours, the swimming velum disappeared, marking the transition to benthic juveniles. Furthermore, phylogenetic analyses using the COX1 partial gene and 13 mitochondrial protein-coding genes revealed significant genetic divergence of the Chuuk population compared to previously reported populations from New Caledonia and French Polynesia. Our newly assembled mitochondrial genome (17,664 bp) notably differed from existing unverified sequences. This genetic distinctiveness suggests that the Chuuk Atoll population may represent a genetically unique lineage or subspecies (tentatively referred to as *Rochia* aff. *nilotica*). The findings from this study provide essential baseline data for conservation and aquaculture strategies, emphasizing the need for regional management tailored to genetically distinct populations.

# ASSOCIATION BETWEEN GUT MICROBIOME COMPOSITION AND PARASITE INFECTION IN THE MANILA CLAM *Ruditapes philippinarum*: IMPLICATIONS FOR PROBIOTIC APPLICATIONS

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The gut microbiome plays a crucial role in bivalve immunity and resilience against pathogens. The Manila clam *Ruditapes philippinarum* has experienced production declines in Korea due to environmental changes and the protozoan parasite *Perkinsus olseni* infections. This study examined the relationship between gut microbiome composition and *P. olseni* infection across Incheon, Seocheon, and Jeju along the coast of Korea. Incheon had the highest infection intensity (1,454,425 ± 470,709 cells/g tissue), while Seocheon was lowest (310,889 ± 470,709). Amplicon sequencing revealed regional microbiome differences: Alphaproteobacteria dominated Incheon and Seocheon, whereas Mollicutes prevailed in Jeju. Pathogenic bacteriai *Vibrio splendidus* and Rickettsia-like organisms (RLOs) were more abundant in Incheon, aligning with higher infection levels. Conversely, Jeju and Seocheon showed higher proportions of *Mycoplasma* and *Metamycoplasma*, which were negatively correlated with parasite loads. Notably, Mycoplasma may suppress *Perkinsus* growth by competing for similar resources within the host and is more frequently found in healthy clams, suggesting its role as a symbiotic bacterium with potential probiotic applications to improve clam health and disease resistance.

# HIGH DESERT AQUACULTURE WORKFORCE EDUCATION

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The Controlled Environment Agriculture (CEA) program at Santa Fe Community College (SFCC) in New Mexico, USA, trains students to be future technicians for aquaculture and indoor crop production facilities. With a focus on aquaponics, hydroponics and algae cultivation in recirculating aquaculture systems (RAS) Charlie Shultz and Steve Gomez have awarded more than 177 degrees over the past 10 years. They have spawned local businesses including New Mexico's first indoor vertical aquaponics farm and New Mexico's first commercial spirulina farm. The program collaborates with regional, national and international organizations like the Algae Foundation, the Algae Technology Educational Consortium, the Aquaponics Association, World Aquaculture Society, US Department of Energy, US Department of Defense, and many universities. Grants support student training and community support, many coming from the US Department of Agriculture. The facilities include an off-grid 1,100$m^2$ greenhouse with solar energy collectors and battery storage, at-scale hydroponic and aquaponic systems, algae cultivation systems from benchtop photobioreactors to farm scale production raceways, a Biofuels Center of Excellence, rainwater collection and storage, and other greenhouses and indoor production rooms. Students can earn degrees in CEA and Algae Cultivation as well as associated trades degrees like Water Technologies, Water Conservation Technologies, and Water Treatment Operation. Using RAS systems to produce nutritious food throughout the year is crucial in a region with very little freshwater resources like Santa Fe, NM. Aquaponic systems at SFCC operate with a daily recycle rate of >99%, requiring very little water to produce crops continuously. The program and facility have become a model for other schools and communities and provide tours for thousands of visitors each year. As a community college, our students and faculty engage with industry, creating pathways from college into employment.

# PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE OF *ULVA* SPP. IN LAND-BASED RECIRCULATING AQUACULTURE SYSTEMS

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As marine space becomes increasingly limited and inland food systems face sustainability challenges, land-based seaweed cultivation in closed recirculating aquaculture systems (RAS) offers a promising alternative. Within the EU project NOVAFOODIES, we evaluate the feasibility of a closed, land-based *Ulva* spp. cultivation system (Fig. 1) using artificial seawater. The land-based RAS design (Fig. 1) allows for precise control of growth conditions, minimal water exchange, and potential integration with renewable energy sources, making it a model for sustainable production.

The objective of this study is to evaluate the physiological and biochemical performance of two *Ulva* spp. strains with different reproductive traits, cultivated under identical conditions across multiple 14-day cultivation cycles. The cultivation setup consists of two vertical shelving RAS units (~300 liter each), each containing six transparent 60-liter culture tanks (Fig. 1). Sampling is conducted at regular intervals (Day 0, 4, 7, 11, 14) within each cycle to monitor physiological development over time. Key data collected include growth rate, biomass (Fig. 2), yield, photosynthetic efficiency dissolved inorganic nutrient uptake (phosphate, ammonium, nitrate, nitrite), carbon and nitrogen content, total protein content, pigment composition, and specific leaf area. In addition, 24-hour nutrient uptake studies at early and late cultivation stages provide high-resolution data on nutrient dynamics and can be linked to physiological performance.

Preliminary results suggest characteristic changes in growth efficiency and nutrient assimilation across the harvest cycle, providing insight into optimal harvest timing, biomass quality and system efficiency. The findings contribute to the development of scalable and sustainable inland seaweed production systems and highlight the potential of land-based RAS as a viable component for the future of seaweed aquaculture.

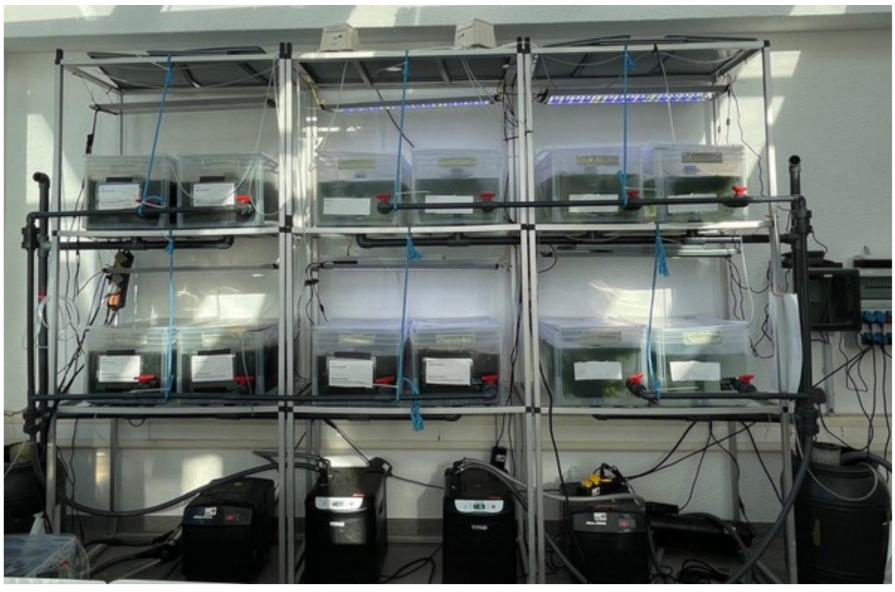

**Fig. 1:** NOVAFOODIES land-based recirculating aquaculture system (RAS) for seaweed cultivation. The setup includes two vertical shelving RAS units, each with six transparent 60 L culture tanks—System 1 (left) and System 2 (right). Two *Ulva* spp. strains with different reproductive traits are cultivated under identical conditions. The system is located at the Centre for Aquaculture Research, Alfred Wegener Institute. Bremerhaven. Germanv. © Hofmann. L.C. 2024

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**Fig. 2:** Fresh harvest of *Ulva* spp. within the NOVAFOODIES land-based recirculating aquaculture system (RAS). The two *Ulva* spp. strains with different reproductive traits, are cultivated under identical conditions across multiple 14-day cultivation cycles. The system is located at the Centre for Aquaculture Research, Alfred Wegener Institute, Bremerhaven, Germany. © Hofmann, L.C. 2024

# PHOTOPERIOD MANIPULATION INDUCES CHANGES OF THE GUT MICROBIOTA IN ATLANTIC COD *Gadus morhua*

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## Introduction

Microbiota is affected by environmental factors, including photoperiod, and this can have a profound effect on host physiology. However, little is known about the influence of sex on light-related changes in microbial composition. Our objective was to investigate sex-specific effects of photoperiod manipulation on gut microbiota in Atlantic cod (*Gadus morhua*).

## Materials & Methods

Juvenile Atlantic cod were divided into two experimental groups in triplicate tanks, which were kept under 24 h continuous light (CL) or 12 h light: 12 h dark (AL) for a period of 6 months. The microbiota of 60 samples collected from gut contents (15 males and 15 females from each photoperiod condition) was characterized by 16S rRNA V3-V4 region amplified on Illumina NovaSeq 6000 sequence (Siriyappagouder *et al*., 2018)"container-title":"Frontiers in Microbiology","volume":"9","abstract":"Establishment of the early-life gut microbiota has a large influence on host development and succession of microbial composition in later life stages. The effect of commensal yeasts - which are known to create a conducive environment for beneficial bacteria - on the structure and diversity of fish gut microbiota still remains unexplored. The present study examined the intestinal bacterial community of zebrafish (Danio rerio. The 16S rRNA sequences were first subjected to a quality control, trimming, merging and chimera removal procedure using the QIIME2 pipeline. Taxonomy was assigned to Amplicon Sequence Variants (ASVs) using the SILVA reference database and the feature table output was imported into Rstudio for further processing using the R package phyloseq  and pctax.

## Results

We identified a core microbiota consisting of 966 ASVs (99% sequence similarity), shared across all individuals. The most abundant taxa at phylum level were Spirochaetota, Bacteroidota and Proteobacteria, which were dominant in all samples. Proteobacteria dominated in both photoperiod groups and consisted mostly of the genera *Aliivibrio*, *Photobacterium*, *Psychromonas* and *Vibrio*. In the female and male groups exposed to CL there was an increase in the relative abundance of *Brevinema* and a concomitant reduction in the proportion of *Mycoplasma* in comparison to samples undergoing AL regime. Beta diversity analysis based on Bray–Curtis dissimilarity and Jaccard index revealed significant differences in community composition between AL and CL groups (PERMANOVA, $p = 0.003$), suggesting a strong effect of photoperiod on the gut bacterial composition in Atlantic cod. Alpha diversity indices (Shannon, Simpson and Chao1) showed significantly higher diversity in males under continuous light compared to their female counterparts ($P < 0.05$), while no significant differences with sex were observed under 12:12 photoperiod. In particular, Lefse analysis showed that Ruminococcoccaceae and Brevinemataceae were enriched in males and females exposed to continuous light, respectively.

## Conclusions

Our results show that photoperiod manipulation induces changes in the gut microbiota of Atlantic cod. Furthermore, higher alpha diversity was observed in males than in females exposed to continuous light. The differential taxon enrichment with sex may have implications in fish physiology, including sexual maturation.

## Acknowledgments

This study has received funding from the Research Council of Norway under the Researcher Project for Scientific Renewal (EPICOD, project No. 336112), the Spanish Ministry of Science and Innovation grant PID2023-146286OB-I00 "Holosex" and FPI contract PRE2022-101795 associated to grant CEX2019-000928-S-20-5 funded by AEI 10.13039/501100011033 (Spain).

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Siriyappagouder, P., Galindo-Villegas, J., Lokesh, J., Mulero, V., Fernandes, J. M. O., & Kiron, V. (2018). Exposure to yeast shapes the intestinal bacterial community assembly in zebrafish larvae. *Frontiers in Microbiology*, 9. https://doi.org/10.3389/fmicb.2018.01868

# IMPACT OF PARTIAL OR COMPLETE REPLACEMENT OF FISH MEAL WITH HIGH-QUALITY MEAT MEAL IN THE DIET OF RED SEA BREAM (*Pagrus major*) ON GROWTH PERFORMANCE AND FEED UTILIZATION

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## Introduction

Fish meal (FM) is often considered as neither eco-friendly nor sustainable. Therefore, feed nutritionists are actively seeking reliable and cost-effective protein sources to substitute for FM. Meat meal (MM) has a lower cost (USD 0.86/kg, USD 1 = 1400 KRW) compared to FM (USD 2.03/kg), and it is classified into low-quality [≤ 75% crude protein (CP)] and high-quality (≥ 80% CP) MM based on their protein content. Among them, high-quality MM has advantages including high CP and lipid content, and year-round availability as a FM alternative in fish diets.

Red sea bream (*Pagrus major*), a carnivorous fish species, is a valuable species of marine fish in Republic of Korea and Japan. Gunathilaka et al. (2023) proved that 50% of FM could be replaced with high-quality MM (CP: 84.2%) without compromising growth and feed consumption when fingerling (initial weight of 50.2 g) red sea bream were fed with a 60% FM-based diet or one of diets replacing 50% of FM with plants, animals, or their blends.

The size (or age) of fish can greatly influence the substitutability of FM by alternative proteins in diets. In general, smaller (younger) fish are less likely to tolerate higher level of FM substitution. However, no study on the substitutability of high-quality MM for FM in juvenile red sea bream diets has yet been performed.

Therefore, this study aims to evaluate the impact of partial or complete FM replacement with high-quality MM in the diet on the growth performance and feed utilization of red sea bream.

## Materials and Methods

The control (Con) diet contained 55% FM (Table 1). In the Con diet, the partial (20%, 40%, 60%, and 80%) or complete (100%) levels of FM were replaced with high-quality MM, referred to the MM20, MM40, MM60, MM80, and MM100 diets, respectively. All experimental diets were formulated to be isoproteic at 51.5% and isolipidic at 14.5%. A total of 450 juvenile red sea bream (7.9 ± 1.85 g; mean ± SE) were distributed into 18 of 50-L flow-through tank (25 fish per tank) with triplicate. All fish were hand-fed to apparent satiation twice a day for 8 weeks. At the end of the 8-week feeding trial, growth performance, feed utilization, blood chemistry, proximate composition, and amino acid and fatty acid profiles were analyzed.

Data were subjected to One-way ANOVA and Tukey's honestly significant difference test using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

## Results

Weight gain, specific growth rate (SGR), and feed consumption of fish fed the Con, MM20, and MM40 diets were significantly ($P < 0.0001$ for all) higher than those of fish fed the MM60, MM80, and MM100 diets (Fig. 1 and Fig. 2). In regression analysis, linear relationships were revealed to be the most suitable model between dietary elevated MM replacement for FM (X) and weight gain ($Y = -0.145434X + 31.6540$, $R^2 = 0.9253$, $P < 0.0001$), SGR ($Y = -0.008442X + 2.9195$, $R^2 = 0.9161$, $P < 0.0001$), and feed consumption ($Y = -0.133145X + 32.5305$, $R^2 = 0.9213$, $P < 0.0001$), respectively. In orthogonal polynomial contrast, weight gain and feed consumption of red sea bream revealed linear ($P = 0.0001$ for both), quadratic ($P = 0.002$ and $P = 0.014$, respectively), and cubic ($P = 0.030$ and $P = 0.019$, respectively) relationships with dietary MM replacement levels for FM, but SGR revealed linear ($P = 0.0001$) and quadratic ($P = 0.001$) relationships with dietary elevated MM replacement for FM. However, blood chemistry, proximate composition, and amino acid profiles of fish were not significantly ($P > 0.05$) influenced by dietary FM replacement with MM.

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**Table 1.** Ingredients and chemical composition of the experimental diets (%, DM basis).

| | Experimental diets | | | | | |
|---|---|---|---|---|---|---|
| | Con | MM20 | MM40 | MM60 | MM80 | MM100 |
| *Ingredients (%, DM)* | | | | | | |
| Fish meal (CP: 73.4%, CL: 10.7%) | 55.0 | 44.0 | 33.0 | 22.0 | 11.0 | |
| Meat meal (CP: 83.9%, CL: 9.9%) | | 9.5 | 19.0 | 28.3 | 37.5 | 47.0 |
| Fermented soybean meal | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 |
| Wheat flour | 17.5 | 18.8 | 20.1 | 21.6 | 23.2 | 24.5 |
| Fish oil | 4.0 | 4.2 | 4.4 | 4.6 | 4.8 | 5.0 |
| Soybean oil | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Others | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| *Nutrients (%, DM)* | | | | | | |
| Crude protein | 51.3 | 51.6 | 51.4 | 51.3 | 51.5 | 51.3 |
| Crude lipid | 14.6 | 14.7 | 14.5 | 14.5 | 14.6 | 14.4 |

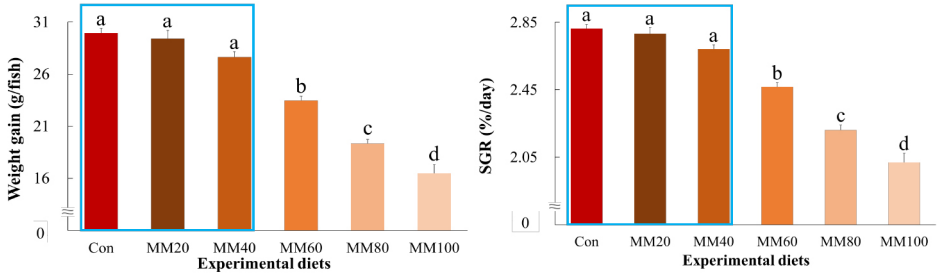


**Fig. 1**. Weight gain (g/fish) and specific growth rate (SGR, %/day) of red sea bream fed the experimental diets ($P < 0.0001$ for all).

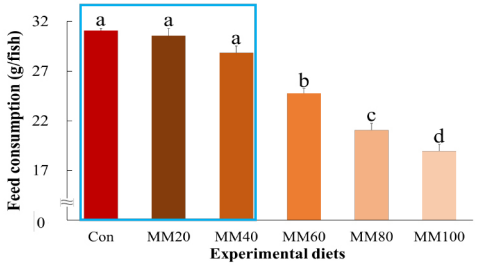


**Fig. 2.** Feed consumption (g/fish) of red sea bream fed the experimental diets ($P < 0.0001$).

## Conclusion

FM up to 40% could be replaced with MM in a 55% FM-based diet without significantly impairing the growth performance and feed utilization of juvenile red sea bream.

## References

Gunathilaka, B.E., Jeong, S., Cho, M., Kim, K., Hur, S., Lee, S., You, S., Lee, S., 2023. Effects of dietary fish meal replacement with alternative protein ingredients and their combinations on growth performance, feed utilization, fillet composition, and biochemical parameters of red seabream (*Pagrus major*). Aquac. Nutr. 2023.

# EFFECTS OF REPLACING FISH MEAL WITH PLANT PROTEIN SOURCES IN DIETS WITH OR WITHOUT JACK MACKEREL MEAL ON THE GROWTH PERFORMANCE OF RED SEA BREAM (*Pagrus major*)

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## Introduction

With stagnant production and high price of fish meal (FM), feed nutritionists are searching for a reliable and affordable source as a FM replacer. Corn gluten meal (CGM) and soy protein concentrate (SPC) are useful plant protein sources as a FM replacer in fish diets. Dietary 50% FM replacement with CGM led to lower growth of red sea bream (*Pagrus major*) than fish fed a 60% FM-based diet when fingerling (initial weight of 50.2 g) fish were fed with a 60% FM-based diet or one of diets replacing 50% of FM by CGM, SPC, meat meal, and chicken by-product meal as a single ingredient, and their combination (Gunathilaka et al., 2023). The authors stressed that the substitutability of CGM for less than 50% of FM should be assessed in the red sea bream diet. However, in that study, dietary 50% SPC replacement for FM achieved comparable growth of fingerling red sea bream to fish fed the 60% FM-based diet. Still, the substitutability of FM with SPC in the juvenile red sea bream diet has been unknown. The size (or age) of fish can influence the substitutability of FM by the replacers in fish diet.

The low-FM diets commonly cause poor palatability, decreased feed consumption by fish, and eventually deteriorate growth. In our previous study (Beak and Cho, 2024), jack mackerel meal (JMM) had the highest attractiveness to juvenile red sea bream among 18 protein sources, and 24% inclusion of JMM instead of FM in diets led to the greatest growth of red sea bream by improving feed consumption.

Therefore, this study investigates the effects of replacing FM with CGM and SPC in diets with or without JMM inclusion on the growth performance of juvenile red sea bream.

## Materials and Methods

A three-way {2 FM replacement source (CGM and SPC) × 2 FM replacement level (20% and 40%) × 2 JMM inclusion (without and with)} ANOVA experimental design was applied. Nine experimental diets were formulated to be isoproteic at 52.0% and isolipidic at 15.0%. The control (Con) diet contained 60% FM. In the Con diet, 20% and 40% of FM were replaced with CGM and SPC without or with inclusion of 24% JMM at the expense of FM, referred to the CGM20, CGM40, SPC20, SPC40, CGM20J, CGM40J, SPC20J, and SPC40J diets, respectively. Six hundred and seventy-five juvenile fish were assigned into 27 of 50-L flow-through tanks with triplicate. All fish were hand-fed to apparent satiation twice a day for 8 weeks. At the end of the 8-week feeding trial, growth performance, feed utilization, blood chemistry, proximate composition, and amino acid and fatty acid profiles were analyzed.

Data were subjected to Three-way ANOVA and Tukey's honestly significant difference test using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

## Results

Weight gain, specific growth rate (SGR), and feed consumption of red sea bream fed the CGM-replaced diets were significantly ($P < 0.001$ for all) higher than those of fish fed the SPC-replaced diets (Fig. 1A). Furthermore, dietary replacements of 20% FM achieved higher weight gain, SGR, and feed consumption in fish compared to those of 40% FM (Fig. 1B). Additionally, weight gain, SGR, and feed consumption of fish fed the all plant protein-replaced diets with JMM inclusion were significantly ($P < 0.0001$, $P < 0.001$, and $P < 0.001$, respectively) higher than those without JMM inclusion (Fig. 1C). Weight gain, SGR, and feed consumption of fish fed the Con diet were significantly ($P < 0.0001$ for all) higher than those of fish fed the CGM40, SPC20, SPC40, CGM40J, and SPC40J diets (Fig. 1D). However, feed utilization, blood chemistry, proximate composition, and amino acid profiles of red sea bream were not significantly ($P > 0.05$) influenced by dietary treatments.

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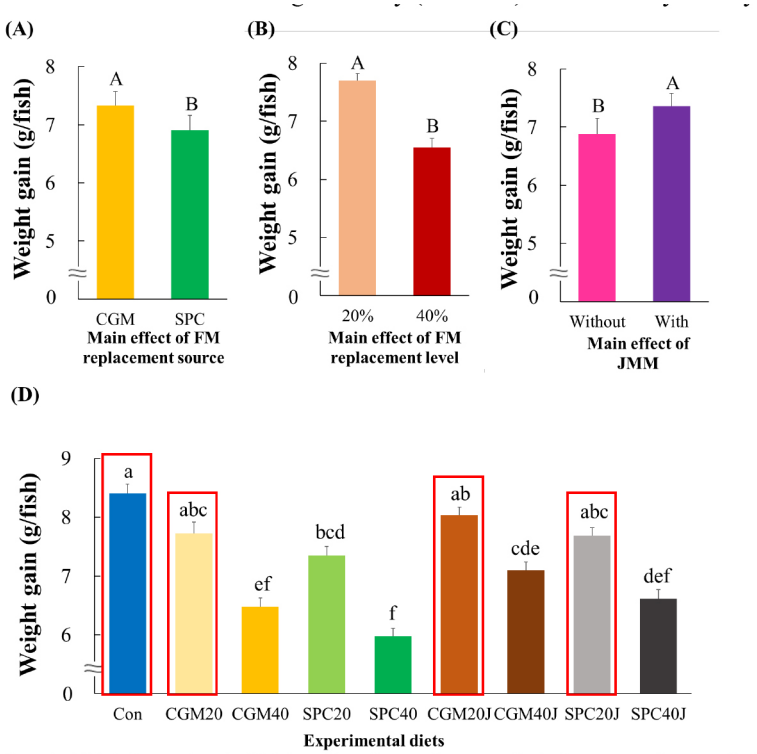


**Fig. 1.** Weight gain (g/fish) of red sea bream fed the experimental diets. The different uppercase and lowercase under numerical values indicate significant differences ($P < 0.05$) based on Three-way ANOVA and Tukey's honestly significant differences test, respectively.

## Conclusion

The CGM-replaced diets achieved better growth performance and feed consumption of red sea bream than the SPC-replaced diets. Moreover, dietary replacements of 20% FM attained better growth and feed consumption of fish than those of 40% FM. Additionally, better growth and feed consumption was obtained in fish fed the all plant protein-replaced diets with JMM inclusion compared to those without JMM inclusion. FM up to 20% could be replaced with CGM with or without JMM inclusion and SPC with JMM inclusion in the 60% FM-based diet of red sea bream without causing significantly negative effects on the growth performance and feed availability.

## References

Baek, S.I., Cho, S.H., 2024. Effects of dietary inclusion of a crude protein source exhibiting the strongest attractiveness to red sea bream (*Pagrus major*) on growth, feed availability, and economic efficiency. Animals 14, 771.

Gunathilaka, B.E., Jeong, S., Cho, M., Kim, K., Hur, S., Lee, S., You, S., Lee, S., 2023. Effects of dietary fish meal replacement with alternative protein ingredients and their combinations on growth performance, feed utilization, fillet composition, and biochemical parameters of red seabream (*Pagrus major*). Aquac. Nutr. 2023.

# MANIPULATION EFFECTS OF JACK MACKEREL MEAL IN LOW-FISH MEAL DIETS SUBSTITUTING FISH MEAL WITH VARIOUS ANIMAL PROTEINS ON THE GROWTH PERFORMANCE AND BIOCHEMICAL COMPOSITION OF ROCKFISH (*Sebastes schlegeli*), AND ECONOMIC ANALYSIS

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## Introduction

Fish meal (FM) has considered an unsustainable and costly feed ingredient, so feed nutritionists are actively seeking a cost-effective and consistently available alternative for FM. Chicken by-product meal (CBM), meat meal (MM), and tuna by-product meal (TBM) are popular a FM replacer in fish feeds. However, low-FM diets often result in reduced palatability and feed consumption by fish, ultimately leading to decreased growth performance. To resolve these issues, manipulation of feed ingredient exhibiting strong attractiveness to a target fish into the low-FM diets is one of the best options.

Therefore, this study aims to evaluate manipulation effects of jack mackerel meal (JMM) exhibiting strong attractiveness to rockfish (*Sebastes schlegeli*) in low-FM diets substituting 25% and 50% FM with various animal protein sources (CBM, MM, and TBM) on the growth and biochemical composition of rockfish, and economic analysis.

## Materials and Methods

A two-way [3 FM substitution source (CBM, MM, and TBM) × 2 FM substitution level (25% and 50%)] ANOVA experimental design was applied. The control (Con) diet included 55% FM. In the Con diet, 25% and 50% of FM were substituted with CBM, MM, and TBM with an additional inclusion of 22% JMM at the expense of FM, referred to the CBM25J, CBM50J, MM25J, MM50J, TBM25J, and TBM50J diets, respectively. All experimental diets were to be isoproteic at 51.5% and isolipidic at 14.5%. A total of 525 juvenile rockfish were randomly distributed into 21 of 50-L flow-through tanks with triplicate. All fish were hand-fed to apparent satiation twice a day for 8 weeks. At the end of the 8-week feeding trial, growth performance, feed utilization, blood chemistry, proximate composition, and amino acid and fatty acid profiles were analyzed.

The economic evaluation of the experimental diets for rockfish was conducted using formulas from the study by Martínez-Llorens et al. (2007): economic conversion ratio (ECR, USD/kg) = feed consumption of fish (kg/fish)/weight gain (kg/fish) × diet price (USD/kg) and economic profit index (EPI, USD/fish) = [final weight (kg/fish) × fish sale price (USD/kg)] − [ECR (USD/kg) × weight gain (kg/fish)].

Data were subjected to Two-way ANOVA and Duncan's multiple range test using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

## Results

Weight gain and specific growth rate (SGR) of rockfish fed the CBM- and TBM-substituted diets were significantly ($P < 0.005$ and $P < 0.004$, respectively) higher than those of fish fed the MM-substituted diets (Fig. 1A). Moreover, dietary 25% FM substitutions achieved significantly ($P < 0.0001$ for both) higher weight gain, SGR, and feed consumption in fish compared to dietary 50% FM substitutions (Fig. 1B). Weight gain and SGR of rockfish fed the Con, CBM25J, TBM25J diets were significantly ($P < 0.0001$ for all) higher than those of fish fed the CBM50J, MM50J, and TBM50J diets (Fig. 1C). Feed consumption of rockfish fed the MM25J diet was significantly ($P < 0.01$) higher than that of fish fed the Con, CBM50J, MM50J, and TBM50J diets. However, the dietary treatments did not significantly ($P > 0.05$) affect biological indices, plasma and serum parameters, proximate composition, and amino acid profiles of rockfish. The highest EPI was observed in the CBM25J diet (Table 1).

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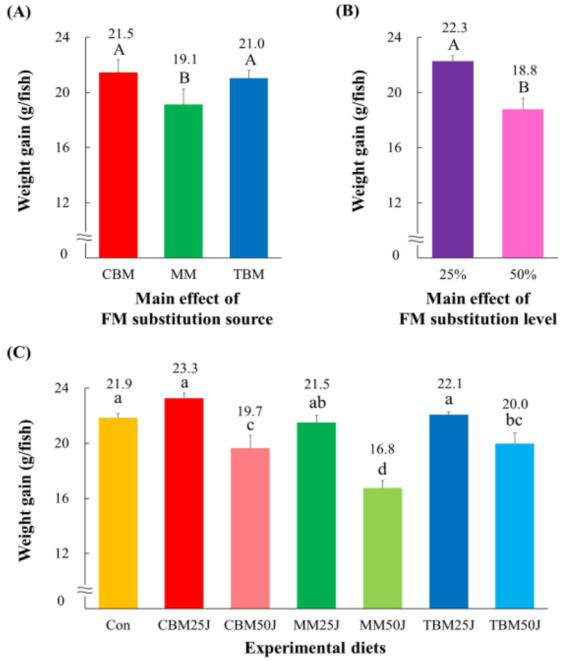


**Fig. 1.** Weight gain (g/fish) of rockfish fed the experimental diets. The different uppercase under numerical values indicate significant differences ($P < 0.05$) based on Two-way ANOVA.

**Table 1.** Economic parameters of the feeding trial.

| Experimental diets | Diet price (USD/kg) | ECR (USD/kg) | EPI(USD/fish) |
|---|---|---|---|
| Con | 1.63 | $1.43 \pm 0.008^{bc}$ | $0.97 \pm 0.008^{ab}$ |
| CBM25J | 1.59 | $1.40 \pm 0.037^{c}$ | **$1.02 \pm 0.013^{a}$** |
| CBM50J | 1.42 | $1.39 \pm 0.043^{c}$ | $0.90 \pm 0.032^{c}$ |
| MM25J | 1.60 | $1.57 \pm 0.063^{ab}$ | $0.96 \pm 0.019^{abc}$ |
| MM50J | 1.44 | $1.63 \pm 0.087^{a}$ | $0.81 \pm 0.016^{d}$ |
| TBM25J | 1.64 | $1.50 \pm 0.014^{abc}$ | $0.98 \pm 0.009^{ab}$ |
| TBM50J | 1.52 | $1.42 \pm 0.029^{bc}$ | $0.92 \pm 0.023^{bc}$ |
| *P*-value | | $P < 0.02$ | $P < 0.0001$ |

The two-way ANOVA results were excluded from the table due to space constraints.

## Conclusion

The CBM- and TBM-substituted diets achieved greater weight gain and SGR compared to the MM-substituted diets. Moreover, dietary 25% FM substitutions achieved greater weight gain, SGR, and feed consumption of rockfish than dietary 50% FM substitutions. Furthermore, 25% FM could be substituted with CBM, MM, and TBM in the 55% FM-based diet with 22% JMM inclusion without deteriorating the growth performance, feed consumption, and biochemical composition of rockfish. Finally, the CBM25J diet produced the highest weight gain and SGR of rockfish, and economic return (EPI) to farmers.

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# IMPACTS AND ECONOMIC ANALYSIS OF JACK MACKEREL MEAL INCLUSION IN LOW-FISH MEAL DIETS SUBSTITUTING 50% OF FISH MEAL WITH HIGH-QUALITY MEAT MEAL ON THE GROWTH PERFORMANCE OF OLIVE FLOUNDER (*Paralichthys olivaceus*)

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## Introduction

The global market value of fish meal (FM) has been consistently increasing because of the rapid growth of aquaculture worldwide. Therefore, efforts are needed to look for an alternative source that is both sustainable and inexpensive for FM in fish feeds. High-quality meat meal (MM), an animal by-product, is considered a suitable FM replacer in olive flounder (*Paralichthys olivaceus*) diet. However, low-FM diet commonly deteriorates its palatability and eventually leads to reduced feed consumption and growth of fish. The incorporation of feed ingredient with strong attractiveness to a target fish into low-FM diets is an effective fish culture technique to improve palatability of low-FM diet and feed consumption by fish.

This study thus aims to evaluate the impacts and economic analysis of jack mackerel meal (JMM), which exhibits strong attractiveness to olive flounder, inclusion in low-FM diets substituting 50% of FM with high-quality MM on the growth performance of olive flounder.

## Materials and Methods

The control (Con) diet included 60% FM (Table 1). In the Con diet, 50% of FM was substituted with high-quality MM, and then 0%, 10%, 20%, 30%, 40%, and 50% of JMM were included at the sacrifice of FM, referred to the $MJ_0$, $MJ_{10}$, $MJ_{20}$, $MJ_{30}$, $MJ_{40}$, and $MJ_{50}$ diets, respectively.

Data were subjected to One-way ANOVA and Duncan's multiple range test using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

## Results

Weight gain of olive flounder fed the Con, $MJ_{40}$, and $MJ_{50}$ diets was significantly ($P < 0.02$) greater than that of fish fed the $MJ_0$ and $MJ_{10}$ diets (Fig. 1). Specific growth rate (SGR) of fish fed the Con, $MJ_{30}$, $MJ_{40}$, and $MJ_{50}$ diets was significantly ($P < 0.02$) greater than that of fish fed the $MJ_0$ and $MJ_{10}$ diets.

In regression analysis, linear relationships were shown to be the most suitable model between inclusion levels of JMM (X) in low-FM diets substituting 50% of FM with MM and weight gain ($Y = 0.026534X + 14.0555$, $R^2 = 0.5486$, $P < 0.0001$) and SGR ($Y = 0.002625X + 2.6140$, $R^2 = 0.4800$, $P < 0.001$), respectively. In orthogonal polynomial contrast, weight gain and SGR of fish revealed linear ($P = 0.0019$ and $P = 0.0036$, respectively) relationships with dietary inclusion levels of JMM in low-FM diets substituting 50% of FM with MM. Feed consumption of fish fed the MJ40 diet was significantly ($P < 0.001$) higher than that of fish fed the $MJ_0$, $MJ_{10}$, and $MJ_{20}$, diets. However, the dietary treatments did not significantly ($P > 0.05$) influence feed utilization, plasma and serum parameters, proximate composition, and amino acid profiles of olive flounder. The EPI of the Con and $MJ_{50}$ diets was significantly ($P < 0.04$) higher than that of fish fed the $MJ_0$, $MJ_{10}$, and $MJ_{20}$ diets (Table 2).

## Conclusion

Incorporation of 20%–50% JMM into low-FM diets substituting 50% of FM with high-quality MM attained comparable weight gain, SGR, and feed consumption to fish fed a 60% FM-based diet. Linear relationships between weight gain and SGR of olive flounder vs inclusion levels of JMM in low-FM diets were the most suitable model in regression analysis. Finally, the $MJ_{50}$ diet was the most recommendable treatment to farmers because of the highest EPI.

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**Table 1.** Ingredients and chemical composition of the experimental diets (%, DM basis).

| | Experimental diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | Con | $MJ_0$ | $MJ_{10}$ | $MJ_{20}$ | $MJ_{30}$ | $MJ_{40}$ | $MJ_{50}$ |
| *Ingredients (%, DM)* | | | | | | | |
| Fish meal (CP: 66.9%, CL: 14.5%) | 60.0 | 30.0 | 24.0 | 18.0 | 12.0 | 6.0 | |
| Meat meal (CP: 85.6%, CL: 8.5%) | | 22.4 | 22.4 | 22.4 | 22.4 | 22.4 | 22.4 |
| Jack mackerel meal (CP: 72.8%, CL: 9.8%) | | | 6.0 | 12.0 | 18.0 | 24.0 | 30.0 |
| Fermented soybean meal | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Wheat flour | 20.5 | 25.8 | 25.5 | 25.2 | 24.9 | 24.6 | 24.3 |
| Fish oil | 2.0 | 4.3 | 4.6 | 4.9 | 5.2 | 5.5 | 5.8 |
| Others | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| *Nutrients (%, DM)* | | | | | | | |
| Crude protein | 51.3 | 51.8 | 52.1 | 52.3 | 52.7 | 53.1 | 53.2 |
| Crude lipid | 11.3 | 11.4 | 11.4 | 11.4 | 11.2 | 11.4 | 11.5 |

A total of 525 juvenile (initial weight of 4.2 g) fish were randomly distributed into 21 of flow-through tanks with triplicate. All fish were hand-fed to apparent satiation twice a day for 8 weeks. At the end of the 8-week feeding trial, growth performance, feed utilization, blood chemistry, proximate composition, amino acid and fatty acid profiles were analyzed. The economic evaluation of the experimental diets for olive flounder was conducted using formulas from the study by Martínez-Llorens et al. (2007).

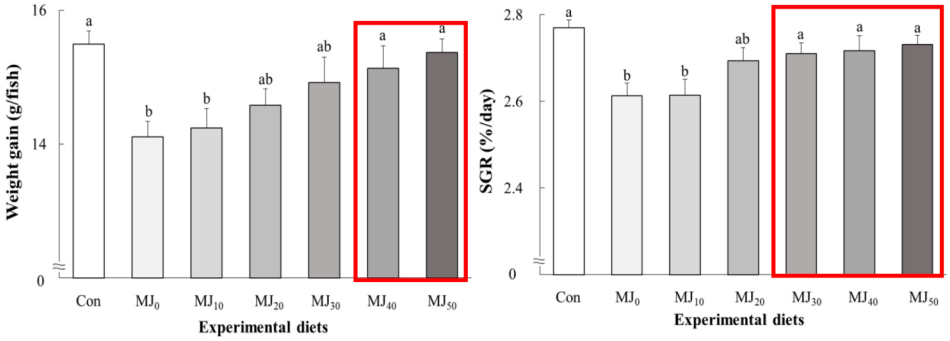


**Fig. 1**. Weight gain (g/fish) and specific growth rate (SGR, %/day) of olive flounder fed the experimental diets.

**Table 2.** Economic parameters of the feeding trial

| Experimental diets | Diet price (USD/kg) | ECR (USD/kg) | EPI (USD/fish) |
|---|---|---|---|
| Con | 1.72 | $1.66 \pm 0.025^{a}$ | $0.63 \pm 0.007^{a}$ |
| $MJ_0$ | 1.40 | $1.39 \pm 0.030^{b}$ | $0.60 \pm 0.007^{c}$ |
| $MJ_{10}$ | 1.42 | $1.40 \pm 0.036^{b}$ | $0.60 \pm 0.009^{bc}$ |
| $MJ_{20}$ | 1.45 | $1.45 \pm 0.042^{b}$ | $0.61 \pm 0.009^{bc}$ |
| $MJ_{30}$ | 1.47 | $1.45 \pm 0.044^{b}$ | $0.62 \pm 0.014^{abc}$ |
| $MJ_{40}$ | 1.50 | $1.49 \pm 0.034^{b}$ | $0.63 \pm 0.011^{ab}$ |
| $MJ_{50}$ | 1.53 | $1.49 \pm 0.015^{b}$ | **$0.64 \pm 0.007^{a}$** |
| *P*-value | | $P < 0.002$ | $P < 0.04$ |

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Martínez-Llorens, S., Moñino, A.V., Tomás Vidal, A., Salvador, V.J.M., Pla Torres, M., Jover Cerdá, M., 2007. Soybean meal as a protein source in gilthead sea bream (*Sparus aurata* L.) diets: effects on growth and nutrient utilization. Aquac. Res. 38, 82–90.

# PHYSIOLOGICAL AND MOLECULAR INSIGHTS INTO TRANSPORT STRESS IN SEA BASS: TOWARDS NON-INVASIVE CORTISOL MONITORING

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**Introduction**

The welfare of farmed fish is a growing concern, driven by both increased mortality and reduced productivity linked to stress, as well as ethical considerations. Transportation of fish, a necessary practice in aquaculture, represents a significant challenge by exposing fish to multiple stressors. Assessing welfare depends on understanding stress responses, and physiological and molecular markers provide crucial insights into an animal's state. Cortisol, a key indicator of hypothalamic–pituitary–interrenal axis (HPI) activity, is extensively studied in aquaculture to assess fish stress. This hormone serves as a reliable indicator of acute stress in plasma. However, the blood collection procedure itself can induce stress, necessitating the identification of less invasive matrices for cortisol measurement (Sadoul & Geffory, 2019). Consequently, this study will assess cortisol correlations across plasma, skin mucus, scales, and water in European sea bass (*Dicentrarchus labrax*) following an acute transport stress. The aim is to identify reliable, less invasive, or non-invasive stress markers, with findings supported by molecular markers from HPI-related tissues (brain, pituitary, and head kidney).

**Materials and methods**

Following a one-week acclimation period, European sea bass juveniles, fasted overnight, were transferred into 10 L plastic bags saturated with oxygen (density: 30 kg/m$^3$) and transported for 4 hours by a transport van. Immediately after transport, half of the transported fish were sampled (transported group, TRANSP) while the rest were returned to their original tanks, to serve as a recovery group (RECOV), with sampling conducted 24 hours later. Non-previously disturbed fish were sampled at each sampling point as controls of transported (CTRL1) and recovery groups (CTRL2). Plasma, skin mucus, scales, and water samples were collected for cortisol analyses. Additionally, the brain, pituitary, and head kidney were extracted for molecular studies.

**Results and discussion**

Transported fish exhibit increased cortisol levels in plasma, skin mucus, scales, and water, confirming cortisol's efficacy as an acute stress marker in all analysed matrices. Notably, the strongest positive correlation (rho=0.8) was observed between scales and mucus, suggesting a similar reflection of cortisol concentration, likely due to their proximity. Moderate yet strong correlations were observed between water and mucus (rho=0.56), water and scales (rho=0.54), mucus and plasma (rho=0.52), and scales and plasma (rho=0.51). Therefore, both mucus and scales may be useful matrices for evaluating cortisol levels in fish due to their strong relationship with plasma levels, as previously reported in other studies (Samaras et al., 2021; Franco-Martinez et al., 2022). The presence of cortisol in water and its positive correlation with mucus and scales reinforces the idea that cortisol is released into water via mucus or skin, supporting the notion that levels in the water reflect those in external body matrices. The weakest correlation, though still positive, was noted between water and plasma (rho=0.38), likely due to their greater physiological distance. Therefore, mucus and scales show strong correlations with each other and with plasma, indicating their potential as non-invasive biomarkers for systemic cortisol levels. While water cortisol is a valid measure, it is a less direct indicator. This may be due to a delayed release of excess cortisol into the external environment after plasma levels normalise post-stressor. Thus, water cortisol can reflect a stressful event in the animal's recent past, even if the acute stressor is no longer present.

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On the other hand, transcriptional changes following transport stress revealed a consistent and significant molecular response across all examined tissues. In the brain, the transported group showed a significant upregulation of *hsp70* and *crfbp*, suggesting activation of cellular stress pathways and *crf* modulation. Conversely, glucocorticoid receptors *gr1* and *gr2* were downregulated in transported fish, suggesting altered sensitivity or feedback to manage prolonged or intense stress. A similar response was observed in the pituitary, with an increase of *hsp70* and a decrease of *gr1* expression in transported fish. All these gene expression changes in both brain and pituitary returned to baseline within 24 hours post-transport, indicating physiological recovery. Surprisingly, *pomca* expression in the pituitary did not rise after transport but showed a significant temporal increase after 24 h of transportation in both CTRL2 and RECOV fish. A similar, but inverse, trend was noted for *gr2* expression. This suggests additional regulatory factors beyond transport stress, potentially influenced by the cumulative fasting period experienced by the animals. In the head kidney, *hsp70, il10*, and *cxcr4* were significantly upregulated in transported fish, reflecting the activation of immune regulatory mechanisms to control the stress response. A comparable pattern, although without significant differences, was observed for *star* expression, which enhances the metabolism of cholesterol, the precursor of cortisol. These findings collectively demonstrate a robust endocrine-immune response to transport in European sea bass, with partial recovery at the transcriptional level in the post-transport phase.

**Conclusions**

Transporting sea bass triggers a coordinated tissue-specific neuroendocrine and immune stress response at both physiological and molecular levels. Marked changes in the expression of genes associated with stress (*hsp70*, *crfbp*), glucocorticoid regulation (*gr1*, *gr2*), and immune modulation (*il10*, *cxcr4*) were observed in the brain, pituitary, and head kidney. These molecular shifts, along with increased cortisol levels after transport, reflect the activation of systemic stress and immune pathways, which partially reverse during recovery. Finally, to address the need to assess animal welfare in such scenarios, the strong correlations found between cortisol levels in plasma, scales, skin mucus, and even water suggest that these matrices offer effective and less stressful alternatives for monitoring cortisol in fish.

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**Acknowledgments**

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# ASSESSING VACCINATION AS A STRESS MODEL IN RAINBOW TROUT AND EUROPEAN SEA BASS

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## Introduction

Vaccination is an indispensable tool in aquaculture, serving as a critical prophylactic measure against infectious diseases that can significantly affect fish health and welfare, leading to substantial economic losses for the industry (Kumar et al., 2024). Despite its benefits, the vaccination process itself presents a suite of stressors, both acute and chronic, that can negatively impact the physiological status of farmed fish. These stressors range from handling and injection to the inflammatory response triggered by the vaccine adjuvant. Therefore, it is imperative to understand and mitigate these stressors to optimise fish welfare, enhance vaccine efficacy, and promote the sustainability of aquaculture production (Ma et al., 2019). This research focuses on characterising vaccination as a quantifiable and relevant stress model within aquaculture systems. We investigated the physiological and metabolic responses to dip vaccination in two commercially significant species: rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*). Plasma levels of energy metabolites and stress markers were assessed in both species at baseline and at different time points within 24 hours post-vaccination. The goal is to identify reliable biomarkers to improve fish health and aquaculture productivity.

## Materials and methods

The vaccination trials were conducted at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) in Portugal. Juveniles of rainbow trout and European sea bass were allocated in triplicates into two separate systems, acclimated for one week at an initial stocking density of 4.5 kg/m³. Environmental conditions were kept constant for each species: sea bass at 20°C and 35 ppt salinity, and rainbow trout at 16°C and 0 ppt. Before the dip vaccination, baseline samples (pre-challenge) were collected. Subsequently, fish from each system were netted and transferred to smaller tanks with strong aeration, containing a commercially available vaccine solution for the vaccine group or only water for the sham group, and remained there for 60 s. After exposure, fish were returned to their original tanks. Post-treatment samples were collected at 1, 6, and 24 hours. Plasma samples served for the comprehensive analysis of stress and metabolic biomarkers, while the pituitary gland and brain were employed for molecular analyses.

## Results and discussion

Principal Component Analyses (PCA) performed on metabolites in sea bass showed that the first two components explained 68.8 % of the variance, with glucose and triacylglycerides (TAG) being the main contributors. The results suggest that time is a more influential factor than vaccination in sample separation. Specifically, cortisol levels increased in both the vaccinated and sham groups 1 hour post-vaccination, being only significant in vaccinated fish. The rapid increase in cortisol levels after vaccination, followed by a subsequent decline, indicates that both the procedure and the vaccine itself contribute to the overall stress response, establishing cortisol as a reliable biomarker for measuring this effect. Glucose exhibited a similar pattern to cortisol, but remained higher in the vaccinated group compared to the sham group after 6 hours before returning to baseline at 24 hours. Lactate levels, influenced by time and vaccination, increased significantly 1 hour post-vaccination in both groups, being more pronounced in vaccinated fish. These findings indicate an immediate acute stress response to the procedure, with lactate serving as a more reliable marker for differentiating the immediate effects of the vaccination procedure, while glucose discriminates the longer-term effects or recovery. Cholesterol levels in the vaccinated group were significantly lower 24 hours post-vaccination, which could be related to its role as a precursor for cortisol synthesis. TAG levels also showed a significant decrease, but temporal variations between vaccinated and sham fish suggest differential regulation in the activation

of TAG synthesis depending on the source of stress. Finally, preliminary gene expression analysis in sea bass revealed that the stress response in the pituitary gland and brain was regulated primarily by time rather than by vaccination. However, specific vaccination-related effects were observed, including changes in the expression of *pomca* and *gh* in the pituitary, and *crf* in the brain.

In contrast, in rainbow trout, more metabolites (glucose, TAG, and proteins) participate in the separation of the first two principal components, which together contribute to explaining 75.5 % of the total variance. Again, the effect of time is the most influential factor, regardless of the effect of vaccination. Cortisol and glucose appear to have the greatest influence 1 hour post-vaccination, while cholesterol, proteins, and TAG levels are better indicators of the recovery response. After the vaccination procedure, cortisol levels increased in both groups, although a statistically significant increase was only found in the vaccinated group after 6 hours. Glucose and lactate levels showed a similar pattern, increasing after the procedure, with the vaccinated group displaying significantly higher levels of both parameters compared to the sham group at 6 hours post-vaccination. Again, the increase in cortisol suggests that the vaccination procedure induces an acute stress response, although it was slower than in sea bass. Regarding metabolic parameters, TAG levels increased over time, showing significant differences after 24 hours, while cholesterol began rising after 6 hours post-vaccination. The opposite metabolic response observed in sea bass suggests physiological and metabolic differences between the species, likely due to distinct adaptations to stress and the use of energy reserves.

**Conclusions**

The vaccination procedure induced a significant and species-specific physiological stress response in both fish species. Cortisol and glucose levels consistently increased following the vaccination procedure, confirming their use as reliable biomarkers for the overall acute-stress response. Glucose and TAG were particularly useful for monitoring the longer-term recovery phase. Ultimately, the results demonstrated that time, rather than the vaccination itself, is a more influential factor in regulating the overall physiological and molecular responses. This suggests that the stress generated by the entire procedure, including handling and vaccine administration, has a significant impact on the animal.

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# A DISH WITH NO FISH: HOW DHA-FREE MICROALGAE CAN BE AN ALTERNATIVE FATTY ACID SOURCE IN DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

Aquaculture is a rising sector and to maintain increasing fish production, an equal increase in feed production is necessary (Tacon, 2020). Especially fish meal and oil play an important role as protein and omega-3 fatty acid source in fish nutrition (Tocher, 2015). However, regarding sustainability and limiting wild fish stocks, alternative feed materials are currently of high interest. EPA (20:5 n-3) and DHA (22:6 n-3) are known as important long-chain polyunsaturated fatty acids (LC-PUFA) derived from fish in human nutrition as well as important fatty acids for the fish health itself. As fish are not able to produce omega-3 PUFA de novo, these fatty acids must be supplied by their diet. Despite to most saltwater fish species, rainbow trout are capable of biosynthesising the LC-PUFA EPA and DHA if enough ALA (18:3 n-3) as precursor is provided (Henderson and Tocher, 1987). Microalgae as primary producers in the marine food chain bring a broad offer of different nutrients and fatty acid profiles. The microalgae *Tetraselmis chui* showed a good potential as fatty acid source (Simon et al., 2024). It contains no DHA but low amounts of EPA and additionally offers ALA and SDA (18:4 n-3), an intermediate of the n-3 biosynthesis. In this study different n-3 PUFA profiles with increasing algae inclusion were evaluated for their effect on this biosynthetic pathway.

## Material and Methods

To test if *T.chui* could be an suitable fatty acid source in fish diets, a feeding trial with rainbow trout was conducted to evaluate growth performance and fatty acid utilisation of the fish. Fish oil was partially (0 %, 33 %, 66 %, 100 %) substituted by the microalgae resulting in decreasing DHA concentrations in the diets. Additionally, two diets (fish oil 0 % and 100 %) were enriched with ALA by adding linseed oil. Triplicates of groups of 20 fish per diet were fed daily with 1.9 % of their body weight for 12 weeks following a 2-week faecal collection period to determine digestibility.

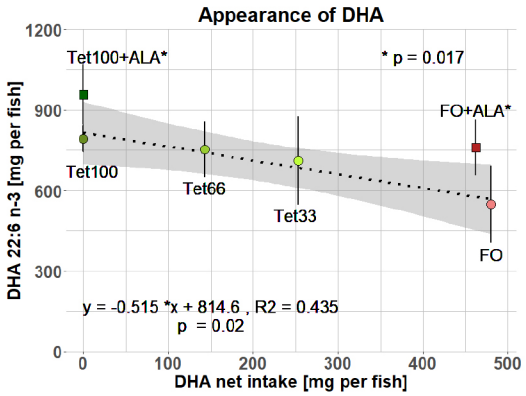


*Fig. 1: Appearance of DHA in rainbow trout whole body after 12-week feeding with different dietary DHA intake*

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**Results and Discussion**

The algae diets resulted only slightly in lower growth performance of the fish, although showing a lower digestibility in accordance with other studies of microalgae (Cardinaletti et al., 2018; Sarker et al., 2020). No significant effect was observed regarding health parameters of the fish as well as no effect in the whole-body nutrient composition. However, with increasing algae inclusion the DHA contents in the fish are decreasing as fatty acid composition of the fish mostly mirrors diet composition. But following the n-3 pathway an increasing proportion of biosynthesised DHA (Fig. 1, circles) can be seen, underlining the ability of rainbow trout to produce this fatty acid from precursors (Sargent et al., 2002). The addition of ALA did not influence the growth performance but increased the DHA production (Fig. 1, rectangles).

**Conclusion**

*T.chui* showed great potential as alternative fatty acid source in fish nutrition although lacking in DHA. Rainbow trout could compensate this lack by DHA biosynthesis and the increase of dietary ALA additionally promoted the DHA production. Understanding the physiological fatty acid pathways in the fish is essential for future feed formulations as sustainable use of important fatty acids is needed.

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# TRANSGENE DELIVERY STRATEGIES TO INTEGRATE CATHELICIDIN DISEASE RESISTANCE GENE IN CHANNEL CATFISH GENOME BY CRISPR/CAS9 KNOCK-IN SYSTEM

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**Introduction**

CRISPR/Cas9-based gene knock out in mammalian cells particularly in teleost has proven to be very efficient with mutation rates, but precise insertion of exogenous DNA or gene knock-in via the homology directed repair pathway remains elusive. While microinjection is more routinely used to introduce CRISPR components into fish embryos, it is labor-intensive and time-consuming, as embryos need to be injected individually. A common alternative to microinjection is electroporation, which uses a rapid and high-voltage electric pulse to deliver DNA into embryos. Here, we utilized microinjection, single electroporation, where CRISPR components were co-delivered with the donor DNA into fertilized eggs, and double electroporation, where both sperm and fertilized eggs received the CRISPR components and donor DNA.

**Materials and methods**

The microinjection experiment had three treatments pertaining to the three DNA constructs carrying cathelicidin gene, a disease-resistant gene derived from American alligator *Alligator mississippiensis*, designated in this study as: dsDNA-UBI-Cath for dsDNA construct driven by zebrafish ubiquitin promoter, dsDNA-BA-Cath for dsDNA construct driven by carp β-actin promoter and plasmid-UBI-Cath for plasmid DNA with zebrafish ubiquitin promoter. Three different concentrations for each of these constructs were used for microinjection namely 10, 20, and 40 ng/μL. For the electroporation experiment, there were three treatments: plasmid microinjection (20 ng/μL plasmid DNA construct), single electroporation and double electroporation. Integration rates were calculated as the number of positive individuals detected by PCR in a replicate or treatment/total number of individuals in the same replicate or treatment *100. Expression of cathelicidin transgene in positive P1 individuals was determined by reverse transcription (RT)-PCR and quantitative real-time PCR (QPCR) analyses.

**Results**

As shown in Table 1, highest integration rates were found using microinjection, both in dead fry with plasmid Ubi as donor DNA (61.5%) as well as in alive fingerlings with dsDNA Ubi donor (31.8%). Moreover, electroporation also proved to be an efficient method to deliver transgene as revealed by 44.4 % integration rate in dead fry and 12.5 % in alive fish using single electroporation technique.

**Discussion**

We succeeded in integrating with high efficiency an exogenous cathelicidin gene into chromosome 1 of channel catfish genome. Effective design of a donor template incorporating the zebrafish ubiquitin promoter, presence of a 250-bp homologous arms at each end of the construct and a sgRNA designed to cleave at the exact insertion site, all contributed to increased homology directed repair (HDR) rate leading to precise integration of the transgene construct into the chromosome 1 of channel catfish genome. The 61.5% integration rate of plasmid Ubi is comparable to the insertion rate of 60 to 90 % using ssDNA donor templates in human cell lines (Sakuma et al., 2016; Aird et al., 2018) and mouse models (Gu et al., 2018). Microinjection remained more effective than electroporation in delivering the transgene construct into the embryos of channel catfish. But electroporation also proved to be an efficient method for transgene delivery. Integration rates were also higher in dead fry than in fingerlings, indicating either off-target effects, pleiotropic effects or lethal overexpression. Additionally, a sensitive area of the genome may have been targeted, although our hypothesis was that this would not be a sensitive area.

Overall, we have successfully generated a CRISPR knock-in transgenic channel catfish carrying cathelicidin gene using microinjection and electroporation strategies.

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**Table 1**. Integration rates of cathelicidin gene constructs in dead fry and surviving fingerlings that were microinjected and electroporated using CRISPR/Cas9 system.

| Delivery Method | Donor DNA construct | Dead fry | | | Alive fingerlings | | |
|---|---|---|---|---|---|---|---|
| | | Total analyzed | Positive fish | Integration rate (%) | Total analyzed | Positive fish | Integration rate (%) |
| Microinjection | dsDNA β-actin | 30 | 6 | 20.0 | 46 | 4 | 8.7 |
| | dsDNA Ubi | 31 | 6 | 19.3 | 22 | 7 | 31.8 |
| | Plasmid Ubi | 13 | 8 | 61.5 | 17 | 3 | 17.6 |
| Single Electroporation | Plasmid Ubi | 9 | 4 | 44.4 | 24 | 3 | 12.5 |
| Double electroporation | Plasmid Ubi | 25 | 5 | 20.0 | 20 | 2 | 10.0 |

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# DEVELOPMENT AND VALIDATION OF A 70K SNP GENOTYPING ARRAY FOR ATLANTIC HALIBUT (*Hippoglossus hippoglossus*)

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**Introduction**
Genetic improvement in Atlantic halibut has been limited by the lack of comprehensive genomic tools. To address this, we developed a mid-density SNP genotyping array for this species [1]. This array supports genomic selection and other advanced genetic applications, facilitating improved breeding outcomes in halibut aquaculture.

**Materials and Methods**
Whole genome sequencing (WGS) data from 40 individuals were used for SNP discovery. Following rigorous filtering [2], 70,824 SNPs were selected and used to design a custom Affymetrix Axiom array. This array was validated on 1152 samples using the Axiom Best Practices Workflow. Genotyping accuracy, SNP performance metrics, and gender determination probes were evaluated to assess array robustness.

**Results**
Sequencing of 40 individuals generated over 8 million variants, including 6,224,375 SNPs and 2,131,436 INDELs. Following stringent quality control filtering, 70,824 high-confidence SNPs were selected for inclusion on a custom Affymetrix Axiom array, including 697 loci informative for sex determination, as females are generally preferred by producers due to their superior growth rate and larger final body size. The array achieved an average sample call rate of 99.64%, and the majority (84%) of SNPs were classified within the top-performing Polymorphic High Resolution (PHR) category. Comparison of array-based genotyping results with WGS-derived genotypes in 36 individuals showed a concordance rate of 99.0% for PHR SNPs [1]. Approximately 95% of SNPs exhibited a minor allele frequency (MAF) greater than 0.05, confirming the array's utility for population-level genomic studies (Figure 1).

The extent and decay of linkage disequilibrium (LD) across the genome was also evaluated, revealing an average $r^2$ of 0.083 for SNPs within 0.1 Mb. LD decayed rapidly with increasing physical distance, dropping to an average $r^2$ of 0.017 at 10 Mb, suggesting high recombination rates and broad genetic diversity within the analyzed populations. Chromosome-specific differences in LD patterns were observed, with chromosomes 3, 5, 7, 8, 13, and 14 exhibiting relatively elevated LD levels [1]. These results indicate the SNP array is well-suited for genome-wide association studies (GWAS) and genomic selection applications in Atlantic halibut. The array also incorporated sex-associated SNPs and structural markers located in and around the gsdf gene, demonstrating robust performance in differentiating genetic sex.

**Discussion and Conclusion**
The development of a robust 70K SNP genotyping array represents a major advancement in the genomic toolkit available for Atlantic halibut, enabling high-resolution analyses across the genome. The array demonstrated excellent technical performance, with high call rates, strong concordance with whole-genome sequencing data, and informative marker distribution across the genome [1,2]. These features support its suitability for applications such as genome-wide association studies (GWAS), pedigree reconstruction, and genomic selection in breeding programs [3].

The array's ability to capture population-wide genetic variation, as evidenced by minor allele frequency distribution and linkage disequilibrium (LD) analyses, underscores its value for studying genetic diversity and structure. LD decay patterns were consistent with high recombination rates and a relatively diverse breeding population [1], indicating that the array offers sufficient marker density for fine-scale mapping and selection strategies.

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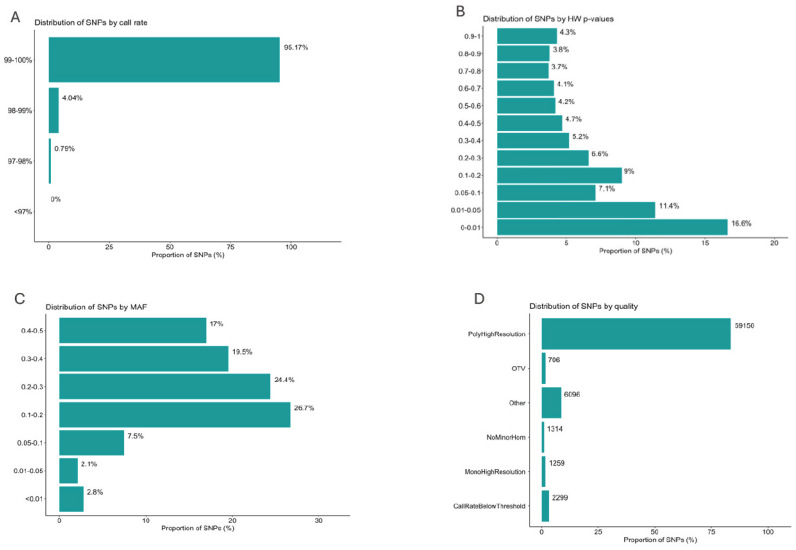


Figure 1. Summary of SNP metrics based on 70,824 SNPs in 1138 Atlantic halibut samples, showing SNP call rate, Hardy-Weinberg equilibrium, minor allele frequency, and SNP classification.

In addition to genome-wide applications, the inclusion of sex-determination markers targeting the gsdf region and associated structural variants provides a practical tool for assigning genetic sex. This is particularly relevant in aquaculture, where female halibut are often preferred due to their faster growth and larger size at harvest [1,4]. The ability to genetically identify sex early in development offers breeders a non-invasive and reliable method to support population management and production efficiency.

Collectively, the performance and design of this mid-density SNP array provide an important resource for advancing genomic research, selective breeding, and management of Atlantic halibut in both commercial and conservation contexts.

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# PRE-ONGROWING OF ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) WITH COMMERCIALLY AVAILABLE DIETS IN EUROPE

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## Introduction

The development of artificial diets for rearing Atlantic Bluefin Tuna (ABT) is crucial for several reasons. Overall, it will support sustainability, economic viability, and reduced environmental impact in tuna aquaculture.

Availability of a commercial feed program for ABT on the market is mandatory for successful ABT aquaculture. The commercial feed should be at least as good performing as feeding based on bait fish, sustain the fish health and performance under aquaculture conditions, and open the opportunity to reduce the amount of marine ingredients in ABT feeding and reduce the trophic level of ABT aquaculture.

A full feeding program should offer solutions for all development and age stages in the aquaculture lifecycle of ABT, starting from weaning over grow-out to brood-stock diets.

The trial presented here aimed to continue the very successful weaning trials with readily available commercial weaning diets, performed in 2023 and presented at WAS conference in 2024, where fish were successfully fed a commercial diet up to 150g. The target of the trial presented here, was to rear juvenile ABT from about 150g onwards to the end of the growth season in winter with a readily available Skretting diet, developed for Tuna.

## Material & Methods

Over 16.5 weeks, an ABT feeding trial was conducted at the Instituto Oceanográfico de Murcia (IEO). A group of 1175 ABT juveniles with a mean weight of 20g at 60 days post hatch (dph) was split into two groups. One group of 520 fish was fed artificial diets from Skretting (Comm. feed group), the other group of 625 fish was re-weaned from artificial diets to bait and continued fed bait fish (sardine and squid) (Bait group). The detailed feeding regime is listed in Table 1.

For the first three weeks of the trial both groups were fed Gemma Silk (GS) a commercial starter diet for marine fish.

Then from about 85dph onwards the Comm. feed group was transferred to Magro diet (M). The Bait group was transferred at the same time (85dph) to natural diets consisting of pieces of sardine and small squid.

Table 1.: Feeding regime of the two ABT groups in the trial.

| Group | 60-84 dph | 85-95 dph | 96-122 dph | 123-152 dph | 153-175 dph |
|---|---|---|---|---|---|
| Comm. feed | GS | M 3mm | M 6mm | M 7mm | Feed paste |
| Bait | GS | Bait | Bait | Bait | Bait |

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For the Comm. feed group, the size of the pellet was successively increased. At 96 dph the fish were changed from 3mm to 6mm Magro, at 123 dph to 7mm Magro. For every size change, there was a period of at least 3 days of co-feeding with both feed sizes.

At 153 dph (after 3 month) the Comm. feed group was changed to a wet diet produced on site, consisting of hydrated Vitalis feed, enriched with Ori-N3 DHA enrichment.

**Results & Discussion**

Both groups showed unusual and very high mortality rates. By the end of the trials (mid-December) both groups were left with less then 25 fish per group and a mortality rate of 95.9 and 96.7% for the Comm feed and bait fish group respectively. These abnormal mortalities were attributed to a previous bacterial infection, not fully overcome.

What became apparent already in the first and second feeding period from 60 and 85 dph onwards is the lower growth performance of the Comm. feed group. While the Bait group reached already 125g at 91 dph, the Comm. feed group only had 68g. The lower performance of the Comm feed group continued until 153dph. During all this time the specific growth rates (SGR) of the Comm. feed was 0.9% lower compared to the bait group.

Only during the last feeding period from 153-175 dph the Comm. feed group had a 0.4% higher SGR. However, by the end of the trial the size difference between the two groups was huge. While the Comm. feed group reached a mean weight of 452g by day 175 post hatch, the Bait group was more then double the size with 987g.

This preliminary trial delivered two main results:

1. The group offered the commercial feed Magro was much worth performing compared to the bait fish reference. In addition, the fish showed also a lower performance then experienced in the previous trials, where the fish were fed the Magro diet up to 150g. The Magro feed was optimized for high growth rate with corresponding high protein levels in the feed.
2. When the fish from the Comm. feed group were offered a different diet, the performance increased.

Additional data evaluation on the whole-body composition of the fish from both groups is planned, however, the results are not available yet. It is expected that these analysis may reveal a specific nutrient deficiency in the Comm feed group.

The preliminary results lead to the following conclusions:

- Offering ABT a high protein diet during the critical growth period of 50g onwards, seems to the wrong strategy.
- Based on the assumption that starting from 50g onwards, ABT is developing its internal temperature regime, a different feeding strategy, less focusing on growth and rather on key physiological changes in the fish might be more promising.
- Additional trials and more in-depth research are needed to understand the specific requirements of ABT in this vulnerable life stage, to develop an adequate feeding strategy for Atlantic Bluefin Tuna.

# FUTURE OF OFFSHORE-BASED CIRCULAR ECONOMY MARITIME PLATFORM FOR SUSTAINABLE FISH PRODUCTION

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## Introduction

This initiative envisions a scalable, offshore circular economy maritime platform for sustainable fish production, centered on a fully enclosed fish farming pool that ensures biosafety and environmental protection by isolating operations from the marine environment. Integrating all stages of aquaculture from hatchery to logistics, the platform includes living quarters and supply docks and is powered mainly by renewable energy. It tackles key challenges such as energy autonomy, nutrient recycling, and global scalability, offering a resilient, sustainable alternative to coastal aquaculture systems.

## Vision

The envisioned platform redefines offshore aquaculture by introducing a circular, autonomous supply system operating entirely at sea. Central to this vision is the use of fully enclosed fish rearing pools that maintain complete separation from the ocean. These systems prevent any interaction with open water, thereby eliminating the risk of pathogen intrusion and ensuring there is no environmental degradation, such as seabed contamination from waste. The design allows for the integration of all stages of production—from hatchery to grow-out, processing, and logistics—into one self-sufficient system. Energy supply is almost entirely renewable, leveraging solar, wind, and wave energy, and supported by redundant backup systems for operational security.

## System Architecture

The system architecture is based on a modular network of platforms designed for various operational functions. Production modules house enclosed liner pools, which support controlled exchange of seawater or freshwater, regulated feed content, and oxygenation. These are complemented by living and working modules that accommodate permanent on-site personnel. Docking stations enable the seamless arrival and departure of vessels for supply and transport. The entire infrastructure is powered by a hybrid renewable energy system composed of wind, wave, and solar sources. Backup diesel generators ensure the protection of livestock and maintain essential systems during unfavorable conditions.



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**Environmental Protection & Biosafety**
At the heart of the environmental protection strategy is the use of an enclosed offshore liner pool that operates without any direct exchange with surrounding sea water. This design creates a robust barrier against pathogens and external contamination, ensuring biosecure conditions for fish farming. Furthermore, internal wastewater treatment systems and the ability to monitor and adjust water parameters result in minimal environmental footprint. The platform protects natural ecosystems by avoiding contact with marine life and eliminating waste discharge into the ocean. These features also significantly reduce the need for antibiotics or medications, enabling organic aquaculture practices.

**Development Priorities**
Key development priorities focus on building scalable, energy-autonomous offshore platforms that integrate diverse systems into a coherent whole. This includes advancing biosafe aquaculture technologies that ensure environmental isolation while enabling controlled rearing conditions. The integration of energy, supply logistics, housing, and food processing into a single marine facility is a complex challenge, demanding interdisciplinary innovation. Additionally, minimizing waste through a circular economy approach—such as the on-site generation of fertilizers and complete resource utilization—remains a central goal.

**Global Relevance**
This offshore platform model presents a globally relevant solution to the increasing strain on coastal environments. By moving sustainable fish production into offshore zones, it alleviates pressure on nearshore ecosystems and supports global food security. The design is particularly suitable for deployment in regions facing climate stress or lacking adequate coastal infrastructure. Its adaptability allows for use even in tropical zones by employing technologies for controlled oxygenation and thermal regulation. Overall, it sets a precedent for next-generation marine food systems that are sustainable, resilient, and ecologically responsible.

# GREEN ENERGY FOR BLUE MARKETS – SUSTAINABLE AQUACULTURE WITH A FUTURE

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**Management Summary**
Aquaculture operations are undergoing structural transformation. Rising energy costs, increasing regulatory pressure, and growing demands for sustainability are putting conventional operating models under serious strain. At the same time, the deployment of maritime floating solar systems is opening new solution spaces. Integrating these technologies significantly improves environmental performance, reduces operational costs, and enhances infrastructure resilience. This trend is rapidly evolving into a new industry standard—independent of any single manufacturer—and represents a key pillar for the future viability of global aquaculture.

**Vision**
A hybrid energy model for aquaculture facilities that combines fluctuating renewable sources with energy storage systems—transforming diesel generators from primary energy sources into backup solutions. This system is embedded in a scalable infrastructure that can be integrated into existing operations without additional permitting, ensuring long-term compliance with regulatory sustainability requirements.

**Opportunities**
Aquaculture facilities stand to gain energy autonomy and improved supply security by integrating floating solar systems that directly feed into onboard battery storage. Diesel generators can still be used but with significantly reduced runtime, boosting resilience to supply disruptions. Operational costs drop dramatically due to reduced fuel use and maintenance, resulting in annual savings in the five-digit euro range per site, even with fluctuating $CO_2$ taxes. Sustainability improves markedly, enabling compliance with EU decarbonization standards and offering enhanced market appeal through certifications and green labels. Integration is straightforward, using existing infrastructure and avoiding new permitting procedures. This builds on proven technology from freshwater solar applications. Insurance benefits further strengthen the business case, as floating systems can be covered by marine insurers and standardized components reduce operational risks—offering greater investment security to all stakeholders.

**Threats**
Despite these opportunities, many aquaculture sites still rely heavily on diesel generators, resulting in high $CO_2$ emissions and risks of marine pollution. This dependence threatens long-term viability as environmental regulations become stricter. Electricity from diesel generators is costly and inefficient. It also exposes facilities to changing fuel prices and possible fuel shortages, making it hard to plan for the future. Regulatory pressure is mounting, with EU directives demanding tangible decarbonization and energy efficiency efforts. Non-compliance may lead to exclusion from key markets and subsidy programs. Moreover, traditional aquaculture operations that rely on diesel are increasingly facing criticism from both environmental groups and society. At the same time, consumer demand is shifting toward more transparent and sustainable production systems, putting additional pressure on industry to adapt. Without innovation in energy use, these facilities risk losing public trust and market relevance, potentially leading to stagnation or decline in support.

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# NURSERY CULTURE AS A SOLUTION TO HIGH SPAT LOSSES IN NEW ZEALAND'S GREENSHELL™ MUSSEL INDUSTRY

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New Zealand's Greenshell™ mussel (*Perna canaliculus*) industry is one of the most inefficient mussel aquaculture industries in the world. The industry harvests over 350 billion spat each year to seed its farms, yet only a fraction of these (< 1%) are retained in production from seeding through to harvest, representing an enormous constraint on growth and production. This presentation will provide an overview of research that has been carried out to develop alternative approaches to nursery culture of Greenshell™ mussels to reduce spat losses and improve production efficiency. It will include results from trials seeking to adapt commonly used bivalve nursery culture systems (FLUPSYs) to mussels and provide insights from studies that aimed to identify the nutritional requirements of Greenshell™ spat so that cost-effective alternatives to live phytoplankton for land-based nurseries can be developed. The presentation will conclude by outlining the next steps required to commercialize these approaches to nursery culture, which will facilitate the transformation of the Greenshell™ industry.

# INITIAL INSIGHTS INTO THE POTENTIAL FOR A SCALLOP *Pecten novaezelandiae* AQUACULTURE INDUSTRY IN NEW ZEALAND

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New Zealand's aquaculture industry is currently dominated by three key species – mussels, oysters, and salmon – and as such, there is growing interest in diversifying the sector. Over the past few decades, wild scallop populations throughout the country have been depleted, resulting in several regional fisheries closures. This decline in wild populations combined with the cultural significance of this species makes it a promising candidate for aquaculture in New Zealand. This presentation will provide an overview of the results from an initial study seeking to determine the feasibility of scallop aquaculture in the Coromandel, in northern New Zealand. It will discuss the key factors limiting the development of scallop aquaculture in New Zealand at each stage of production, namely biofouling and mortality, and suggest some approaches to overcoming these challenges. The presentation will then conclude by providing a summary of the workstreams underway from a recently funded project that seeks to build on the initial results and overcome these challenges to support the development of a scallop industry in New Zealand.

# DIFFERENCES IN THE MINERAL COMPOSITION OF THE TWO MOST COMMONLY FARMED FISH SPECIES IN THE ADRIATIC SEA

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## Introduction

Global aquaculture production reached a record high of 130.9 million tons in 2022, of which finfish accounted 65%. Predominant finfish products in Mediterranean countries include European seabass (*Dicentrarchus labrax* L.) and sea bream (*Sparus aurata* L.), which are valued for their high protein and mineral content and contribute to the increase in annual per capita consumption. In this study, the proximal mineral composition of farmed sea bass and gilthead sea bream from the Adriatic Sea was determined over a one-year period to assess possible species-specific differences in mineral content ($p < 0.05$).

## Materials and methods

Seven representative individual samples per species were analyzed to determine the eight minerals (Na, Ca, K, Mg, Zn, Fe, Cu and P). They were quantified (mg/kg) using flame atomic absorption spectroscopy (200 Series A4 with SPS 4 Auto-Sampler, Agilent Technologies, USA). Normality was tested using the Shapiro-Wilk test and homogeneity of variance using the Levene test. As all minerals showed homogeneity of variance ($p > 0.05$), with the exception of phosphorus, the Welch test was used for this mineral. Due to deviations from normality, the Mann-Whitney U-test was used for zinc and phosphorus, while the Student's t-test was used for all other minerals.

## Results

No statistically significant differences in mineral content were found between the two species ($p > 0.05$), indicating a comparable mineral profile and general richness in essential minerals in both species (Table 1). These results are consistent with previous studies and confirm the comparable nutritional value of these two economically important marine species and the variability in their composition throughout the year.

# ADULT BLACK SOLDIER FLIES (IMAGINES, *Hermetia illucens*) AS A REPLACEMENT FOR FISH MEAL IN DIETS FOR PACIFIC WHITELEG SHRIMP, *Penaeus vannamei*

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## Introduction

Large-scale insect farming holds significant potential for transforming biogenic waste into high-value protein for use in animal feed and human food. The black soldier fly, *Hermetia illucens*, is one of the most cultured insects worldwide. Imagines, the adult life stage of the insect, are only cultivated for reproduction and are arising as novel and unused by-product of industrial black soldier fly larvae production (Soetemanns et al., 2020). Utilization of this side stream as feed ingredient for high value species like *Penaeus vannamei* would reduce waste, create by-product value, and promote a circular economy. In this work, an in-depth chemical analysis of meal prepared from the adult black soldier fly was made and the apparent digestibility of key nutrients was determined *in vivo* in *P. vannamei*. A consecutive growth trial was conducted to assess effects of replacing fish meal with imago meal on weight gain, feed utilization, digestive enzyme activities, and immunological parameters of *P. vannamei*.

## Materials and methods

Dead adult black soldier flies were collected from a local insect farm, dried, and ground to homogenous meal (< 500 µm). Composition of gross nutrients, amino acids, fatty acids, selected minerals, chitin and, cholesterol were determined. Full-fat and partially defatted imago meals were used in a digestibility trial with juvenile *P. vannamei,* using a reference and test diet with yttrium as an inert marker. Based on these findings, experimental diets replacing 50% (I50), 75% (I75), and 100% (I100) of the fish meal content with defatted imago meal were formulated on digestible protein and energy levels. Experimental diets were used for an acceptability trial and a 40-day growth trial with juvenile shrimp (5.4 ± 1.0 g). At the end of the growth trial, digestive enzyme activities were measured in crude extracts made from shrimp midgut glands. Glucose, protein, total hemocyte counts, phenol-, and prophenoloxidase activities were measured in the hemolymph. Shrimp muscle and midgut glands were analyzed for dry matter, lipid, and protein content.

## Results and discussion

Full fat imago meal contains 52% protein, 36% lipid, and moderate levels of chitin (5%). Considerable amounts of amino acids are present with an essential amino acid index of 1.8, referring to the ideal protein for penaeid shrimp (NRC, 2011). Cholesterol and copper were detected at moderate concentrations of 2.8 $g \cdot kg^{-1}$ and 16 $mg \cdot kg^{-1}$, respectively. Defatting the imago meal (IM) reduced the lipid content to 23% and increased protein levels to 60%. Results of the digestibility trial showed, that defatting significantly increased the bioavailability of protein and energy ($p < 0.05$). This is likely related to the low tolerance of shrimp to high levels of dietary lipids and the high content of saturated fatty acids present in imago meal (NRC, 2011; Glencross et al., 2002). However, protein and energy apparent digestibility remained moderate in defatted IM with approximately 60% and 53%, respectively. The acceptability of the defatted IM containing diets was reduced, leading to significantly lower apparent feed intake of the I75- and I100-diets during the growth experiment ($p = 0.001$). Shrimp growth was reduced in all defatted IM-containing diets ($p = 0.0005$). Defatted IM-containing diets caused lower dry matter contents in shrimp muscle ($p = 0.0136$) and lower lipid contents in midgut glands compared to the control diet ($p = 0.0169$; FIGURE 1).

Survival, digestive enzyme activities, and hemolymph parameters were not affected by the diet, implying no adverse effects of defatted IM on general shrimp health. Based on these findings, defatted imago meal is not suitable to replace dietary fish meal at the levels tested in this study. Lower IM inclusion rates (< 100 $g \cdot kg^{-1}$), more efficient defatting of IM, and addition of attractants are suggested to improve the nutritional quality and palatability of imago meal.

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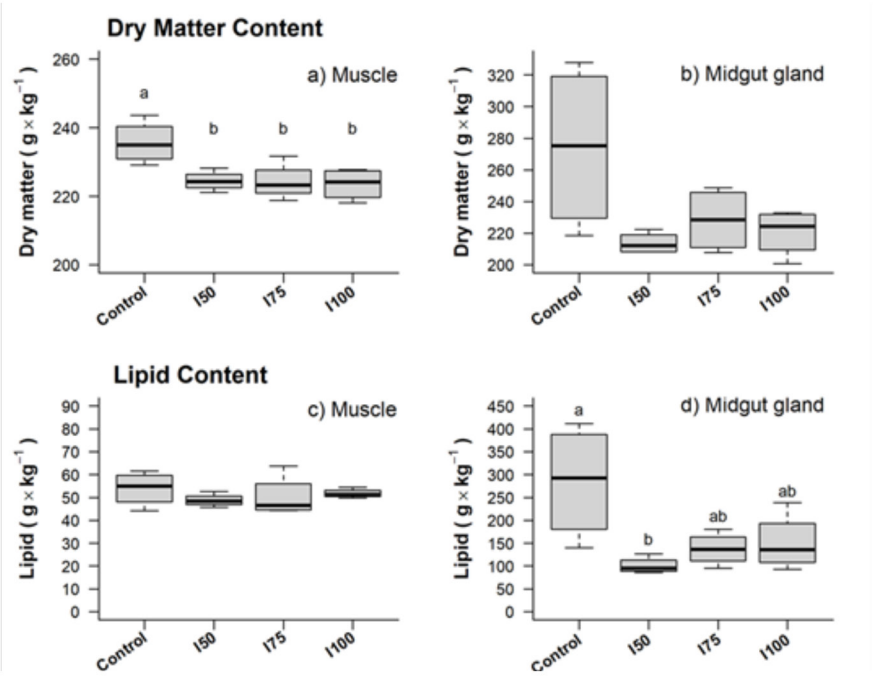


FIGURE 1. Dry matter and lipid contents measured in the muscle (a, c) and midgut gland (b, d) of *P. vannamei* fed a fish meal-based control diet, and three diets in which 50% (I50), 75% (I75), and 100% (I100) of the fish meal was replaced with defatted imago meal (n=4).

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# THE RELATION BETWEEN AQUACULTURE OPERATIONS AND BIOSAFETY IN THE FUTURE OF MARINE FISH FARMING – NEW PRODUCTION STRATEGIES AND PRODUCTION SYSTEMS ON THE RISE

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## Introduction

In 2024 over 100 million Atlantic salmon over 3 grams died in hatcheries and fish farms in Norway. Diseases and parasites are the main root causes, with the most common cause of death being infectious diseases. At the same time, Norwegian aquaculture is largely regulated based on the production of sea lice and how it affects wild salmon. Recently, the authorities put forward a white paper presenting their vision for future regulation of the industry (Meld. St. 24 (2024–2025)) – still largely based on sea lice numbers. Even though it is early to say how this will turn out and ultimately affect the industry, it is reasonable to expect significant changes to production strategies and prevalence of new production systems.

Changes to production strategies and use of production systems following Meld. St. 24 (2024–2025) will potentially affect biosafety. One central consideration is how vessel activities and operations at fish farms are adapted to new regulatory conditions and use of new technology.

## Material and methods

This study investigates how changes to production strategies and production systems for marine farming of Atlantic salmon can affect the biosafety in the industry. The approach is two-folded; i) cases for future scenarios are described and discussed in expert panels, this covers exploring how the industry might look in 20 years and what this means for biosafety, ii) detailed choices for production strategies and production forms are listed and the literature is reviewed for knowledge on the effects of the choices on biosafety, and for possible measures for improvement.

## Results

The study is not yet fully completed, however some preliminary results and details on the structure of the study are available. Case studies are divided into segments for land-based facilities, sea-based facilities and transportation. Choices for production strategies and production forms are further divided into five segments; broodstock, hatcheries, grow-out, slaughter and transport. This is necessary due to the magnitude of choices that can be made from selecting water temperature, geographical placement of facilities, to operations routines and use of stun and bleed vessels.

Extensive literature on risks and measures exists for some choices and alternatives, but there is also a number of choices for which no relevant literature has been identified. This might indicate knowledge gaps with respect to how decision making in aquaculture can affect biosafety. The resulting overview can therefore help guide decision makers in terms of informing them about the risk/consequences related to choices they make.

Further results will be finalized in time for the presentation at Aquaculture Europe 2025.

# PROTEOMIC INSIGHTS INTO TAURINE-INDUCED CHANGES ON EUROPEAN SEA BASS MYOTUBES

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**Introduction**

European sea bass (*Dicentrarchus labrax*) is one of the most commercially valuable species in the Mediterranean aquaculture. However, the use of highly substituted diets for European sea bass is hampered due to the high carnivorous habits of this species. The non-protein amino acid, taurine, has been previously reported as a potential dietary supplement in fish aquaculture, enhancing both growth and antioxidant capacity (D. Montero et al., 2023; P. Salze and Allen Davis, 2015). Here, to better understand the signalling response triggered by taurine in muscle, proteomic and phosphoproteomic analyses were carried out on cultured myotubes from European sea bass.

**Methodology**

European sea bass juveniles (10 ± 2 g of body weight) provided by Culmarex (Mallorca, Spain) were maintained in our indoor facilities at the University of Barcelona. Muscle cells were extracted from 11-12g of white epaxial muscle from juveniles and cultured in quadruplicate (n=4) for 13 days until myotube development stage (García-Pérez et al., *under review*). Cells were treated 24h with 10mM taurine (showed no effects on cell viability) and protein extracted with 8M urea buffer containing a mix of proteases and phosphatases inhibitors. Samples were reduced, alkylated, and digested with LysC and trypsin. Digested peptides were desalted with reverse phase C18 columns, keeping an aliquot for proteome analysis and using another aliquot for phosphopeptide enrichment by IMAC using Fe-NTA magnetic beads. Data acquisition was performed by nano LC-MS/MS using Evosep One chromatograph coupled to an Orbitrap Eclipse mass spectrometer with DDA methods. MaxQuant software was used to identify and quantify the proteome and phosphoproteome using European sea bass database from Uniprot, whereas Perseus and R software were used for bioinformatic analyses.

**Results and discussion**

We quantified 2643 proteins, of which 267 (10.1%) showed significant changes in abundance between taurine and control groups. Hierarchical cluster analysis of z-score normalized changes in proteome abundance allowed to discriminate between control and treatment groups (Fig.1a). Downregulated proteins upon taurine treatment were enriched in gene ontology biological process terms (GOBP) related to cell development (Fig.1b), while mostly protein translation processes were upregulated. We identified 7259 phosphosites, of which 1179 (16,2%) displayed significant differences in intensities between the two groups (Fig.2). Several targets of the mTOR signalling pathway showed increased phosphorylation upon taurine treatment, such as AKT, 4EBP1, and EIF4EBP1, as well as transcriptional factors (e.g., EIF3A) involved in protein synthesis. These findings support a proteinogenic stimulus of taurine in muscle cells by enhancing mTOR signalling and ribosomal activity, thus indicating its potential as a dietary additive for stimulating muscle growth in European sea bass.

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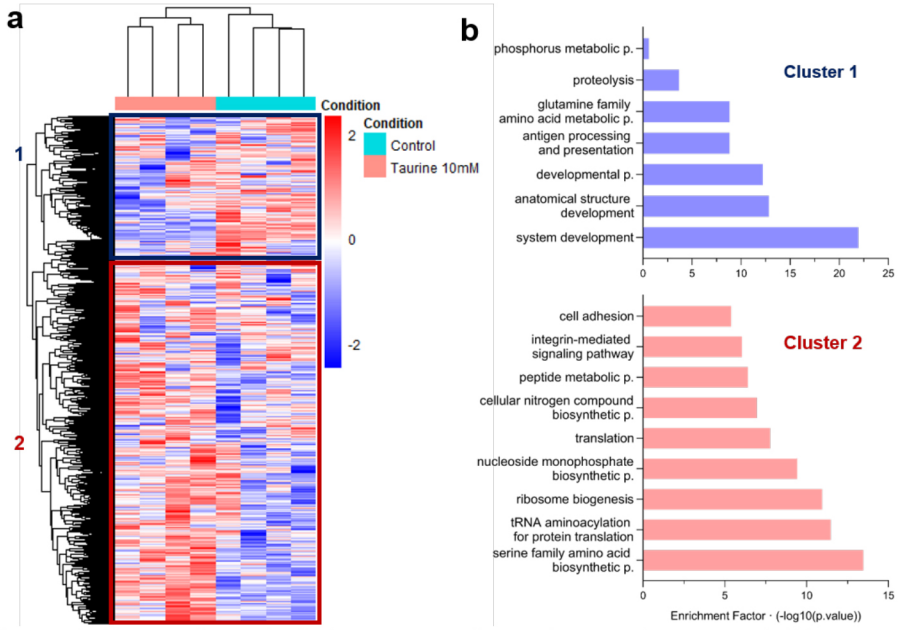


**Figure 1**. Proteomic analysis between taurine and control groups. a) Heatmap and hierarchal clustering of total proteins detected. b) Significantly enriched GOBP terms from main clusters of heatmap.

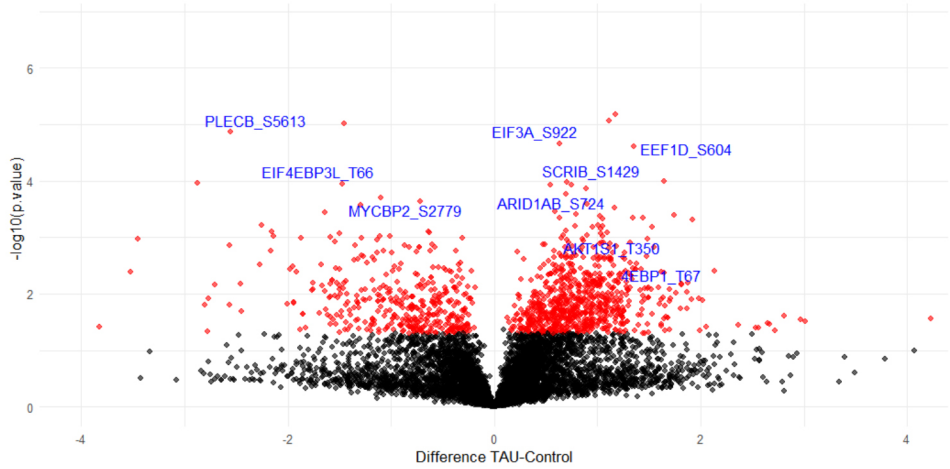


**Figure 2.** Volcano plot comparing taurine and control groups from phosphoproteomic data. (n=3, t-test p-value < 0.05). Highly significant phosphosites involved in mTOR signalling are indicated.

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# FROM RESEARCH TO INNOVATION: PRELIMINARY RESULTS FROM THE THINKINAZUL PROJECT OPEN PATHWAYS TO SAFER AND MORE SUSTAINABLE BABY PRODUCTS BASED ON FISH

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**Introduction**

Carnivorous fish species such as gilt-head seabream (Sparus aurata) require long-chain polyunsaturated fatty acids (LC-PUFA) of the n-3 and n-6 series, including eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and arachidonic acid (ARA; 20:4n-6), crucial both for their optimal health and development and for nutritionally enhancing of products for human consumption (Calder, 2020). EPA and DHA are vital for neurological development during the fetal stage, infancy, and early childhood (Innis, 2007; Lauritzen et al., 2016). Reflecting this, the European Commission has authorized several health/nutritional claims (Regulation (EC) No. 1924/2006).

In parallel, the presence of metal(loid) contaminants in fish-based foods must be minimized due to their carcinogenic and genotoxic potential. Legislative efforts have been made to reduce exposure, including the establishment of maximum limits (Regulation (EC) No. 2023/915). Recent EFSA assessments continue to highlight fish as a major contributor to exposure to arsenic species such as DMA and MMA, particularly in toddlers and elderly populations. Additionally, methylmercury (MeHg) remains a key concern. In contrast, health benefits associated with fish consumption must also be considered.

Ensuring essential nutrient content and minimizing contaminant levels poses a considerable challenge for the food industry. Careful selection of fish species and production methods is therefore critical when designing fish-based products for vulnerable groups. Toddlers' nutrition has become a central concern for the scientific community, the food industry, and general consumers. This study aims to support the development of safer, more sustainable, and nutrient-rich fish products, particularly for toddlers.

**Materials and methods**

As part of an experiment framed within the ThinkInAzul GVA project, three types of feed formulations (n=3, CTRL: fish meal-based diet, PAP: processed animal protein-based diet, and ALT: alternative protein-based diet) were evaluated on fatty acid and mineral profiles of *Sparus aurata* fillets. A Gas Chromatograph coupled to a Flame Ionization Detector (GC-FID) was used for fatty acid detection and semi-quantification, and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used for metal(oid) quantification.

On the other hand, all the published literature on the comparison of metal(oides) in aquaculture fish and wild fish was analyzed using the PRISMA methodology. Thirty-one studies that met the eligibility criteria were selected (Casanova-Martínez et al 2025).

In addition, fish-based pureed baby food, available in Spanish supermarkets and hypermarkets, was evaluated. Product labeling information was collected, including the list of ingredients, nutritional information, and possible health claims.

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**Results and discussion**

*Preliminary background*

According to EU regulations, fish raised with traditional feed is considered a healthy and nutritious option due to its beneficial levels of EPA, DHA, MUFA, and omega-3 fatty acids. The use of the proposed alternative feeds did not compromise these properties and, in addition, had a positive impact on the metal(oid) content of the fish fillets. This ensures that fish-fed with these alternative diets can be considered a suitable option for reducing metal(oid) exposure without compromising their health and nutritional qualities. Thus, the differences between formulations, particularly in As and Hg levels, emphasize the need for careful selection of feed ingredients to minimize metal(oid) exposure in aquaculture. Further research is needed to better understand the metabolism of these compounds and to evaluate the potential health risks associated with their accumulation in farmed fish.

*Literature review*

A higher concentration of metal(oids) such as Hg and As was found in wild fish than in aquaculture fish, although most of the elements were within the limits established for safe consumption by the general population. Even so, it is necessary to reduce the concentrations of these contaminants, especially in foods intended for vulnerable populations. Even so, with the data available in the scientific literature, it is difficult to determine the effect of the fish production system on metal(oid) contamination, since several factors interact in a complex way. The differences found in some research papers are probably not solely attributable to the production system. In addition to species, factors such as size, age, feed type, physiological state, habitat, degree of environmental pollution, diet, or growth rates of aquatic organisms seem to have a greater effect.

*Baby food based on fish and open pathways*

The ingredients of pureed baby foods available on the Spanish market are sources of metal(oids) as they include rice, roots, tubers, and/or fish/shellfish. The study found that fish-based pureed baby foods from the Spanish market account for only 12.04-13.13% of all pureed baby foods, with fish content around 8%. Hake was present in over 50% of these products. Only 28% of products specified the production method, with wild-caught hake frequently identified. This low proportion of fish-based products contrasts with the scientific evidence supporting the need to increase fish consumption during infancy to promote neurodevelopment, particularly in relation to LC-PUFA intake, specifically EPA and DHA. None of the products included nutrition or health claims. Therefore, the development of baby foods with a higher percentage of fish would be desirable to meet the requirements of these claims and ensure benefits for the target population. Careful selection of the fish species to be included in these formulations is recommended. Aquaculture-derived fish could be a suitable option, as it offers more control over the raw material and facilitates regulation of both desirable and undesirable compound levels.

**Conclusions**

This study highlighted the feasibility of using alternative protein-based feeds in aquaculture without compromising the nutritional quality of *Sparus aurata*, particularly in terms of essential LC-PUFA such as EPA and DHA. Moreover, these alternative feeds can contribute to lowering metal(oid) contamination in fish fillets, supporting the development of safer and more sustainable products. The literature review indicated that while wild fish often show higher concentrations of metals(oids), multiple confounding factors complicate direct comparisons with aquacultured fish. Additionally, current commercial fish-based baby foods in Spain underrepresent fish content and lack authorized nutritional claims despite scientific evidence supporting early-life intake of EPA and DHA. Therefore, enhancing fish content in these products -especially from aquaculture sources with better contaminant control- could help meet regulatory health claim criteria and support the nutritional needs of vulnerable populations like toddlers. Further research and regulatory efforts should focus on optimizing both nutrient and contaminant profiles in fish-based foods for early childhood nutrition.

# WHAT A META-ANALYSIS MAY SHOW US ABOUT THE ONTOGENY OF DIGESTIVE ENZYMES IN FISH LARVAE?

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## Introduction

Digestion is a complex multi-level process consisting of physicochemical breakdown and absorption of a large number of organic and inorganic substances entering the body. The key role in this process is played by digestive enzymes, which differ in many parameters: origin (pancreas, stomach, intestine, food objects, symbiotic microbiota, etc.), substrate specificity (proteins, carbohydrates, lipids, etc.), localization (stomach and intestinal cavity, brush border of enterocytes, intracellular), dependence on pH, activators, inhibitors, etc. For fish, the most important enzymes include pepsin, trypsin, chymotrypsin, amylase, lipase, aminopeptidase, maltase, alkaline phosphatase and some others, responsible for the hydrolysis of food substrates in both gastric and non-gastric (except pepsin) fish. Most enzymes (except pepsin) are found in the digestive system of fish in the first days after hatching and can demonstrate several peaks of activity during ontogenesis. Despite the decisive role of these enzymes in the hydrolysis of proteins, lipids and carbohydrates in the digestive tract, the issue of the coupled development of these enzymes in the early ontogenesis of fish remains poorly understood.

The aim of this work is to conduct a meta-analysis of changes in the activity of key digestive enzymes in the early ontogenesis of different fish species.

## Materials and Methods

The data used in this study were obtained from a bibliographic search using the Dimensions application (https://app.dimensions.ai/discover/publicationtool). The following keywords in various combinations were used for this bibliographic search: “trypsin”, “chymotrypsin”, “alkaline proteases”, “fish”, “larva”, “ontogeny”, and “development”. The retrieved articles were carefully screened for descriptions of developmental activity of digestive enzymes, in particular: pepsin (gastric), lipase, trypsin, amylase, chymotrypsin, total alkaline proteases, carboxypeptidase A (pancreatic), N aminopeptidase, alkaline phosphatase, maltase (brush border), leu-ala peptidase and acid phosphatase (intracellular). From the list of analyzed articles (105), a total of 69 studies were selected for correlation analysis (Pearson), including 63 fish species and 2 fish hybrids. The remaining articles were excluded from the analysis due to insufficient number of sampling points for correlation analysis or lack of data on sampling dates.

## Results

Based on the correlation analysis we have found that pepsin (gastric protease) has shown low correlation ($r$ = 0.25-0.50) with trypsin, chymotrypsin, amylase and carboxypeptidase A (pancreatic enzymes) but moderate correlation ($r$ = 0.50-0.75) with alkaline phosphatase (brush border) and strong negative ($r$ = -0.75-1.00) correlation with leu-ala peptidase (intracellular) enzymes. Regarding to intestinal enzymes, we have detected that in 75 and 79% of cases, total alkaline proteases (TAP) correlated moderately ($r$ = 0.50-0.75) or strongly ($r$ = 0.75-1.00) with trypsin and chymotrypsin, correspondingly. Also, the TAP showed a strong correlation ($r$ = 0.75-1.00) with amylase in 80%, lipase in 85%, and carboxypeptidase in 100% of cases.

Using the special data set of papers where the activity of all three enzymes was available (trypsin, chymotrypsin and TAP), we identified four groups of fish based on correlation analysis: 1) a significant positive correlation ($r$ = 0.61–0.97) between chymotrypsin activity and TAP (28.6% of fish species); 2) significant positive correlation between trypsin activity ($r$ = 0.59–0.97), chymotrypsin ($r$ = 0.78–0.97) and TAP (52.4% of fish species); 3) significant positive correlation ($r$ = 0.65) between trypsin activity and OAP (4.8% of fish species); 4) negative correlation between trypsin ($r$ = -0.38 to -0.57), chymotrypsin (r = -0.22 to -0.49) and TAP (14.3% of fish species). Of particular importance is the fact that all species with high activity of

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only chymotrypsin (group 1) and trypsin (group 3) compared to TAP belonged to gastric fish species with a predominance of predatory species. However, all fish species from group 2, in which the effect of both enzymes (trypsin and chymotrypsin) on the TAP was significant and similar, belonged to both gastric and agastric species with different types of nutrition. Group 4, characterized by a negative correlation between the studied proteases, included only Amazon catfish (family Pimelodidae). All correlations will be discussed in the presentation.

**Conclusion**

Due to known limitations related to the impossibility of direct comparison of enzyme activity obtained by different researchers, correlation analysis nevertheless allows one to search for dependencies in such results. This meta-analysis based on a large array of data allows one to identify various relationships between digestive enzymes and to test previously proposed hypotheses.

Meta-analysis of the articles was carried out with the support of the Russian Science Foundation, project No. 23-74-101-01.

# HABITAT CHARACTERISTICS AND PHYTOPLANKTON FOOD SOURCES OF TWO VENERID CLAMS (*Ruditapes philippinarum* AND *Venerupis aspera*) IN JEJU ISLAND, KOREA

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Venerid clams, including the Manila clam (*Ruditapes philippinarum*) and the Venus carpet shell (*Venerupis aspera*), are commercially valuable bivalves. Historically, these two closely related species have shown different distributions based on climatic conditions. *R. philippinarum* is widely distributed in temperate waters, whereas *V. aspera* tolerates warmer, subtropical to tropical regions. As sea surface temperatures rise, both species are expanding its range and both venerid species now coexist on Jeju Island. Our study assessed habitat characteristics, sediment composition, and potential food sources of the two species at three sites in Jeju (Seongsan, Oeido, Seogwipo) to provide baseline data for selective breeding and genetic resource exploration. We found that the distribution of these clams varies by site. Seongsan hosted only *R. philippinarum*, Oeido had only *V. aspera*, and Seogwipo supported both. Sediment analysis showed that Seongsan had a sandy substrate, while Oeido had higher gravel content. Using psbA-targeted metabarcoding, we identified 120 phytoplankton taxa. Seongsan had the highest phytoplankton diversity and was dominated by diatoms, while Oeido and Seogwipo were dominated by cyanobacteria. These findings suggest that the distribution of these clams is influenced by sediment type and food availability, in addition to climate. This information is valuable for future aquaculture and climate change adaptation strategies.

# HARNESSING NATIVE MARINE BACTERIOPHAGES FOR POST-SURGICAL HEALING IN PEARL OYSTERS: A SUSTAINABLE SHIFT FROM ANTIBIOTICS

Dr. Ajai Kumar Sonkar

Pearl oyster farming is a critical industry in marine aquaculture, relying heavily on the surgical implantation of a nucleus and mantle tissue to induce pearl formation. However, this delicate process comes with the risk of post-surgical infections, which can compromise oyster health, survival rates, and the quality of pearls produced. To mitigate the risk of bacterial infection, antibiotics such as oxytetracycline, chloramphenicol, and kanamycin have been used in aquaculture systems. Despite their effectiveness in infection control, the widespread and often indiscriminate use of antibiotics has led to the alarming rise of antimicrobial resistance (AMR), creating both ecological and public health threats. The aquaculture sector, therefore, urgently requires sustainable, effective alternatives to antibiotics, especially in environmentally sensitive systems like pearl oyster farms.

This study aims to present an innovative solution to this growing challenge by exploring the use of naturally derived bacteriophages—viruses that specifically infect and lyse bacteria—as a post-operative treatment in marine pearl oyster culture. Bacteriophages, in contrast to broad-spectrum antibiotics, offer a highly targeted and eco-friendly alternative, with minimal risk of disrupting non-target microbiota and no harmful residue left in the environment. In this research, bacteriophages were extracted from pristine seawater collected from an uncontaminated coastal region of the Andaman Sea, known for its abundant and diverse marine microbial population. The primary objective was to determine the efficacy of these native bacteriophages in promoting post-surgical healing and preventing bacterial infections in the pearl oysters *Pinctada margaritifera* and *Pteria penguin*, which are native to the Andaman Sea.

The experimental design involved a randomized control trial with 400 oysters subjected to surgical implantation of nucleus and graft tissue. The oysters were divided into three groups: a phage-treated group (n=150), an antibiotic-treated group (n=150), and a control group (n=100). The phage-treated oysters were immersed in bacteriophage-enriched seawater for 72 hours, while the antibiotic-treated oysters were exposed to oxytetracycline at 1.5 ppm for the same duration. The control group was maintained in untreated filtered seawater. Several parameters were monitored, including mortality rates, wound healing times, shell closure rates, nacre deposition quality, microbial load at the wound site, and histological tissue analysis.

The results revealed striking differences between the treatment groups. The phage-treated oysters exhibited significantly faster wound healing, with recovery times ranging between 48 and 72 hours, compared to the antibiotic-treated group, which took 72 to 96 hours to recover. Mortality in the phage-treated group was markedly lower (5%) compared to the antibiotic-treated group (12%) and control group (18%). Histological analysis further corroborated these findings, showing reduced inflammation and superior tissue regeneration in the phage-treated oysters. The microbial analysis revealed that phage therapy effectively reduced pathogenic *Vibrio* spp., particularly *Vibrio harveyi* and *Vibrio alginolyticus*, without negatively affecting the beneficial microbial communities essential for oyster health.

One of the key advantages of phage therapy was its specificity—phages only targeted harmful bacteria while leaving beneficial microbes intact. Furthermore, phage therapy demonstrated the ability to self-replicate at the infection site, providing sustained antimicrobial activity without the environmental persistence associated with antibiotic residues. This self-amplifying property ensures a long-lasting effect, making phages an ideal candidate for sustainable aquaculture management.

In contrast, the antibiotic-treated oysters showed moderate healing times and higher levels of pathogen load. This inefficiency could be attributed to the emergence of antibiotic-resistant bacterial strains within the aquaculture system. Additionally, the use of antibiotics raised concerns about potential residual contamination in the final product, which can pose risks to both consumer health and the broader environment.

This study highlights the potential of bacteriophage therapy as a sustainable, environmentally friendly alternative to antibiotics in pearl oyster farming. By reducing mortality rates, accelerating wound healing, and enhancing nacre quality, phage therapy offers a compelling solution to some of the most pressing challenges in the aquaculture industry. Moreover, the study demonstrates the practicality of phage-based interventions, which can be seamlessly integrated into existing pearl farming practices without the need for major infrastructure changes or new regulatory approvals.

In addition to offering a promising alternative to antibiotics in pearl oyster aquaculture, this research opens up avenues for further investigation into the use of phage therapy in other bivalve species and aquaculture systems worldwide. The findings of this study also contribute to the global effort to combat AMR, providing a model for responsible and sustainable aquaculture that aligns with the increasing demand for antibiotic-free seafood production.

# THE NAUI MONITORING AND FORECASTING SERVICE TOOL IN SUPPORT OF AQUACULTURE MANAGEMENT IN THE EUROPEAN NORTHEAST ATLANTIC AND THE MEDITERRANEAN

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NAUI is the latest User Interface Application developed by NOW Systems, designed as a service aggregator to facilitate access to a wide range of met-ocean information in coastal environments. It delivers Early Warning Systems (EWS) and drives forward the concept of smart coasts by enhancing environmental management and data-based decision making.

Several deployments of NAUI are currently supporting diverse coastal services across the European Northeast Atlantic and the Mediterranean Sea, showcasing how coastal monitoring and forecasting data can contribute to the protection and restoration of coastal biodiversity, while also support a more sustainable aquaculture management. Notable examples of services using the NAUI tool in support of local aquaculture include the e3HOPE in the Ebro Delta zone, the BIODIVER-COAST in Galway Bay, and the MOLL-e MALONE in the Ria de Arousa. All of which have been, or are currently, supported by the Copernicus Marine User Engagement Program as national coastal pilot services.

The Ebro Delta, a protected area of high ecological significance for local biota -recognized as a EU Natura 2000 Site, a Natural Park and a UNESCO Biosphere Reserve-, is also a hotspot for aquaculture. Its inner Bays -Fangar and Alfacs- host the highest annual production of mussels (*Mytilus galloprovincialis*) along the Spanish Mediterranean coast, along with some oyster farming. The region is under pressure due to anthropogenic activities (extensive rice agriculture in the delta), and there were significant mussel production decreases in recent years due to high water temperature peaks. The e-3HOPE Service, codeveloped by NOW, LIM/UPC and IRTA, target the area and includes a new operational coastal forecast system that incorporates freshwater inputs from both the Ebro River and the agriculture drainage canals discharging into the inner Bays. This system supports environmental protection and sustained management of aquaculture production in the Ebro Delta. To this aim, the e3HOPE service features a NAUI application that acts as service aggregator, providing visualization of all available coastal forecasts and observational data, including key indicators and an EWS for extreme events mitigation. This NAUI service enables traffic lights-style warning system, indicating local conditions as safe (green), minor concern (orange), or of a major concern (red) for various essential variables. Users can fully customize these alerts by setting specific threshold values for each warning level at each site according to their needs. This local deployment of NAUI in the Ebro Delta, admin by IRTA, eases coastal management, and helps address challenges to the sustainable development of local bivalve mariculture -such as mussel mortality risks linked to marine heatwaves-. It also supports biodiversity conservation in the area, by helping to monitor the suitability of habitat conditions for *Pinna nobilis,* a Mediterranean endemic species listed as Critically Endangered on the IUCN Red List of Threatened Species and whose presence in Fangar Bay is highly sensitive to changes in salinity. Finally, as part of the on-going FLORETHA Project, the e3HOPE service will be upgraded with new modules for coastal flooding and HAB prediction, further enhancing the existing IRTA HAB forecasting system.

**Galway Bay** hosts some of the last remaining populations of native oyster in Ireland, along with multitude of bivalve mariculture production sites. To support sustainable oyster farming in the bay, it is essential to provide timely information on unfavorable environmental conditions, enabling oyster farmers to take preventive actions to protect their stocks. One of the major concerns is the low salinity events, which significantly increase oyster mortality. To address this challenge, Marine Institute partnered

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with NOW Systems are developing the **BIODIVER-COAST Service**, delivered through a NAUI online platform. This service provides EWS for low salinity events in the bay. The warning system has been co-designed with local end users, and salinity thresholds are established based on a combination of oyster farmers' local knowledge, laboratory experiments exposing oysters to varying salinities and temperatures, and model hindcast analysis. In addition to near-real-time alerts based on the 3-day local forecast and the last month hindcast, the NAUI platform provides access to observational data from local monitoring stations, along with maps displaying monthly, seasonal, annual and decadal (2012-2021) statistics for Galway Bay. This comprehensive system allows identifying the spatial distribution of suitable habitat for native oysters, promoting a local sustainable bivalve mariculture and contributing to the preservation of biodiversity in the Bay.

The most recent deployment of NAUI in support of aquaculture is taking place in the **Ria de Arousa**, as part of the **MOLL-e MALONE Project** developed by a consortium grouping USC, NOW Systems, CETMAR, and that target as key stakeholder to the Coastal Observatory of the Xunta de Galicia. Shell fishing is a key activity along the Galician coast, and particularly in the Ría de Arousa, where edible cockle (*Cerastoderma edule*) holds both ecological and economic significance. Sedentary bivalves like cockles are highly vulnerable to abrupt changes in water conditions -especially to sudden extreme variations in salinity and temperature- often triggered by severe weather events. Despite cockles can tolerate short periods of low salinity, prolonged exposure can cause physiological stress and even mortality. Heavy rainfall and sudden river dam discharges, coincident with high tidal conditions, can significantly reduce salinity levels in the Ría, leading to mass mortality events. To address this, a tailored NAUI module to assess cockle mortality risks due to salinity-driven following Parada et al. (2012), is being implemented for all the production zones in the Ria. The service will contribute to enabling data-driven decision-making for shellfish gatherers and authorities, helping to reduce potential economic losses of shellfish harvesters, and to support more resilient shellfish harvesting practices.

The NAUI service aggregator, through its ad-hoc modules and integrated early warning systems associated with the existing local monitoring and forecasting data, leverages the identification of upcoming unfavorable conditions that may negatively impact on the health of bivalve species, such as oysters, cockles, and mussels. It provides short-term forecasts for key parameters, including salinity, temperature and currents, as well as time series from the past month and relevant historical climate data. By offering this integrated, data-driven insight, the NAUI service contributes to addressing critical challenges in achieving sustainable bivalve mariculture, supporting nature-based solutions focused on restoration, and preserving coastal biodiversity, as exemplified through its application in the here showcased services.

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# ASSESSMENT OF THE DEGRADATION OF DIFFERENTLY MANUFACTURED POLYESTER FIBRES IN MARINE-LIKE MEDIUMS: A LAUNDRY RELEASE- BASED SIMULATION

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## Introduction

The washing process of synthetic textiles is one of the main sources of microplastics (MPs) pollution in different environmental compartments, including seawater (1). Polyester (PES) is the most common synthetic polymer in the textile industry and it is characterised by high persistence in the environment since it cannot be easily degraded (2). The fate of PES microfibers depends on both environmental and textile parameters. Photodegradation is considered one of the most common and effective (3). Laboratory simulations of this type of degradation are time-consuming and, thus, accelerated protocols are preferred (3). The aim of this study is the determination of the effect that washing and different manufacturing steps may have on the degradation of PES fibres.

## Materials and methods

Three different types of polyester textiles were used: undyed polyester (U-PES), dyed polyester (D-PES), a polyester commercial garment (G-PES). Fibres from differently manufactured PES fabrics were obtained by cutting the textiles or by washing them in a household washing machine, and collecting the fibres released. Hence, our study provides a realistic approach to examine textile degradation. D-PES and G-PES fabrics were washed under domestic washing conditions (40$^{o}$C, non-bio detergent, 1200 spin) and the fibres were collected from the drainpipe using a 1μm pore PP filter. The degradation of the prepared PES fibres was studied using an accelerated hydrolytic degradation protocol proposed by Sarno et al, 2021 (3), which mimics the processes occurring, for example, in marine environment. Monitoring of the degradation was performed at: $T_0$ (before degradation), $T_1$ (1 hour), $T_3$ (3 hours), and $T_5$ (5 hours). Fibres were analysed by Scanning Electron Microscopy (SEM), micro- FTIR (μFTIR) and Pyrolysis- Gas Chromatography- Mass Spectrometry (Py-GC-MS). The degradation solution used in the simulation was collected and neutralized. For non-target High Pressure Liquid Chromatography- High Resolution Mass Spectrometry (HPLC-HRMS) analysis, the solution was analysed either directly (diluted) or after solid- phase extraction (SPE).

## Results and discussion

Degradation of the samples was evident, with colour and fibre mass loss increasing throughout the process. As far as the fibre weight loss is concerned, washed garment fibres (G-PES) showed higher levels (71% mass loss at T5). SEM analysis showed degradation of all types of PES fibres, already starting at T1. SEM indicated that the washing process did not affect the morphology of the fibres degradation pattern. Py-GC/MS analyses showed a more substantial degradation of the commercial clothing garment fibres released from laundering, with total ion currents (TICs) of D-PES and G-PES at $T_0$ and $T_5$, showing a drastic decrease in the relative intensity of the marker peaks for G-PES samples after 5 h of degradation. Non-target analysis indicated that the release of leachates including dyes, and additives such as 4-nitrophenol, disperse orange 3, phenyldiethanolamine, and acetoacetanilide depended upon textile manufacturing process. Extraction of the degradation solution by SPE before non- target analysis led to the identification of 79 chemicals.

## Conclusion

The fibres released from the commercial clothing garment showed a higher degradation than the fibres from the textile fabric after 5 h. SEM, Py-GC/MS and PES degradation product concentrations in their degradation solutions, supported this result. Different morphology of the clothing garment fibres (napped on the surface), as well as, manufacturing steps and additives could be the cause of their more substantial degradation and the different leachates that were detected by non-target screening and that could reach the sea. Further research is needed to assess the environmental impact of fibres release and degradation, as this may be of great interest for assessing their potential toxicity while preserving ocean biodiversity.

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# FATTY ACID COMPOSITION OF *Gammarus locusta* OFFSPRING FROM WILD AND FROM BROODSTOCK FED MACROALGAL DIETS

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## Introduction

The accelerated growth of marine aquaculture created an urgent need for sustainable and quality ingredients to maintain the performance and nutritional value of farmed species. Marine gammarid amphipods can be an excellent source of essential fatty acids, namely long-chain *n*-3 polyunsaturated fatty acids (LC-PUFA). However, their potential contribution to the formulation of aquafeeds still remains largely unexplored. The present work evaluated the effect of mono-specific macroalgal diets on the fatty acid composition of the offspring of *Gammarus locusta* fed on these macroalgae and compared it with the offspring of wild conspecifics.

## Materials and methods

*Gammarus locusta* breeding pairs were fed one of five mono-specific macroalgal diets (*Ulva* sp., *Fucus* sp., *Laminaria* sp., *Gracilaria* sp., and *Chondrus* sp.), and their broods were collected to determine their fatty acid composition using gas chromatography-mass spectrometry (GC-MS). Subsequently, their FA composition was compared with that displayed by the offspring of conspecifics from the wild.

## Results

The offspring from wild *G. locusta* showed the highest amount of polyunsaturated fatty acids (PUFA) (4.95 ± 0.31 μg $mg^{-1}$ DW), as well as essential fatty acids, namely eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), with an abundance of 2.49 ± 0.21 and 0.87 ± 0.16 μg $mg^{-1}$ DW, respectively. The algal treatment that promoted the highest PUFA content in offspring was *Fucus* sp. (4.46 ± 0.31 μg $mg^{-1}$ DW), but those derived from broodstock fed with *Chondrus* sp. showed the highest DHA levels (0.56 ± 0.10 μg $mg^{-1}$ DW). However, no significant differences in fatty acid composition were found between offspring from broodstock fed with the different mono-specific macroalgal diets. On the other hand, the abundances of stearic acid (18:0) and DHA were significantly higher in the offspring of adults from the wild when compared to those fed on algal-based diets.

## Discussion

These findings suggest that *G. locusta* selectively conserves and biosynthesises essential fatty acids for growth and reproduction, particularly LC-PUFA. However, this process remains to be demonstrated in marine gammarids, as the presence of genes encoding the front-end desaturases necessary to LC-PUFA biosynthesis is yet to be described in their genome. Moreover, this experiment highlighted the importance of maintaining optimal diet properties for gammarid cultivation, as some mono-specific diets may negatively impact their reproductive yield. Nonetheless, all the offspring analysed present a balanced fatty acid composition with potential applications for aquaculture.

## Conclusion

*Gammarus locusta* offspring present a balanced FA profile with potential applications for aquaculture, as they demonstrate a remarkable trophic plasticity by being able to feed on a wide variety of substrates. Further research is needed to fully elucidate the mechanisms underlying trophic upgrading in gammarids. By using both marine and non-marine diets, as well as nutritional programming and selective breeding, it is possible to establish lineages that match or even surpass the fatty acid composition of wild conspecifics.

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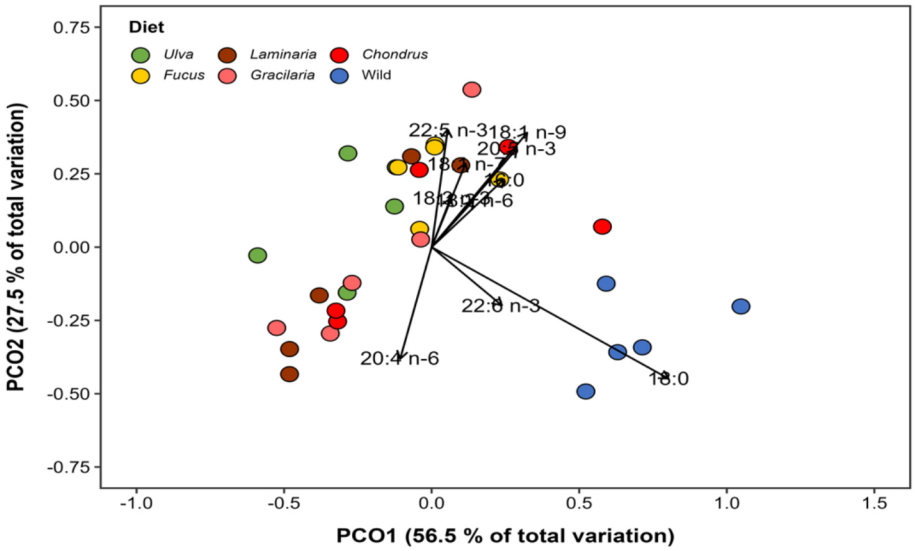


**Figure 1.** Principal Coordinate Analysis (PCoA) illustrating the variation in the fatty acid composition of *Gammarus locusta* offspring from breeding pairs fed with a mono-specific macroalgal diet (*Ulva* sp., *Fucus* sp., *Laminaria* sp., *Gracilaria* sp., and *Chondrus* sp.) or from specimens sourced from the wild. Analysis was restricted to fatty acids in the samples accounting for more than 1% of the total fatty acid abundance.

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# HOUSEMATES IN AQUACULTURE: INTEGRATING *Ulva lactuca* INTO AN IMTRAS WITH *Holothuria arguinensis* FED WITH FISH WASTE

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## Introduction

Integrated Multitrophic Aquaculture (IMTA) stands as a viable avenue to pursue an increase and more sustainable production of marine resources while diversifying commercial output by little to no additional costs. In this regard, sea cucumbers, such as *Holothuria arguinensis*, are deposit feeders that can grow from organic matter deposited at sea floor, making them prime candidates as organic extractors in IMTA systems. However, the circular IMTA concept requires additional agents, like inorganic extractors from the water column like amonia ($NH_3$), nitrites ($NO^-_2$) and nitrates ($NO^-_3$). Macroalgae are strong candidates for this role. The present study sought to determine the viability of producing *Ulva lactuca* in the same recirculating system (RAS) as *H. arguinensis* growing, in turn, in fish waste enriched sediment, in an Integrated Multitrophic Recirculating Aquaculture System (IMTRAS).

## Materials and Methods

To determine the efficacy of *U. lactuca* as an inorganic extractor for aquaculture production, two experimental RAS were put in place, one with the macroalgae (U treatment) and one without (control). Each system was comprised of an individual sump and 3 flat bottom tanks with 50L capacity and substrate enriched with fish waste, provided by Piscicultura do Vale da Lama (Lagos, Portugal). Each tank housed 30 *H. arguinensis* and connected to the respective system's sump. The two sumps were supplied with mechanical and biological filtration, but no protein skimmer. Each sump was then connected to 3 kreisels with 2.9 L capacity, where one system (U treatment) had *U. lactuca* at a density of $1 g \cdot L^{-1}$ and the other system (control) had empty kreisels. Water would then flow into the sea cucumber tanks, closing the circular system. This way, both RAS had the same water volume to better establish comparisons between them. Water quality parameters, mainly nitrogen compounds ($NH_3$, $NO^-_2$ and $NO^-_3$) were measured daily and, once per week, all sea cucumbers had their biometrics registered, such as submerged length, fresh weight, and specific growth rate (SGR). The density ($g \cdot L^{-1}$) of *Ulva* in the U treatment system was also registered in the same weekly interval, to monitor macroalgae growth over time.

## Results

After a period of 6 weeks, while there were no differences between treatments regarding *H. arguinensis* SGR (C = 0.23 ± 0.09 $\% \cdot day^{-1}$; U = 0.24 ± 0.11 $\% \cdot day^{-1}$), sea cucumbers grown with *U. lactuca* presented a higher mean survival rate (U = 97.78 ± 1.92 %) than the ones without (C = 84.44 ± 8.48 %). The inorganic compounds detected in the RAS system with *U. lactuca* were, inversely, at smaller concentrations (U: $NH_3$ = 0.03 ± 0.03 $mgL^{-1}$, $NO^-_2$ = 0.20 ± 0.31 $mgL^{-1}$ and $NO^-_3$ = 1.26 ± 1.42 $mgL^{-1}$) than the one without the macroalgae (C: $NH_3$ = 0.05 ± 0.05 $mgL^{-1}$, $NO^-_2$ = 0.58 ± 0.75 $mgL^{-1}$ and $NO^-_3$ = 2.67 ± 2.18 $mgL^{-1}$).

*U. Lactuca* reared in IMTRAS reached a peak of 2.44 ± 1.99 $g \cdot L^{-1}$ four weeks into the trial, with a maximum SGR of 2.77 ± 0.92 $\% \cdot day^{-1}$.

## Discussion

Sea cucumbers like *Holothuria arguinensis* have continuously demonstrated their aptitude for growth as organic extractors in IMTA settings. However, to reach a circular design and a more sustainable and resilient production system, macroalgae like *Ulva lactuca* are excellent inorganic extractors, mitigating the by-products of animal species in production, thus, improving water quality and showing positive effects on other organisms in the system. Furthermore, the lack of nutritional costs to produce additional trophic levels (commercial sea cucumbers and macroalgae) adds significant value to current production systems.

# DEVELOPMENT AND CHARACTERIZATION OF PLA-BASED ACTIVE FILMS WITH $TIO_2$ NANOPARTICLES AND THYMOL FOR EXTENDING GILTHEAD SEABREAM SHELF-LIFE

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## Introduction

Active and antimicrobial food packaging is a rapidly emerging field, driven by the growing demand for safer, longer-lasting food products, while maintaining food quality, freshness, and safety (Wu et al., 2014). Polylactic acid (PLA), a commercial biodegradable polymer derived from renewable resources, has gained significant attention for food packaging applications due to its transparency, mechanical strength, eco-friendly profile and GRAS (generally recognised as safe) status (Mulla et al., 2021; Sharma et al., 2023). However, neat PLA suffers from brittleness and poor barrier properties, limiting its practical applications (Sharma et al., 2023). To address these limitations, PLA reinforcement with inorganic nanoparticles (NPs), particularly titanium dioxide ($TiO_2$), can enhance mechanical, thermal, and barrier properties, while also providing strong antimicrobial activity through reactive oxygen species generation (Mulla et al., 2021; Nikolic et al., 2021; Nayak et al., 2024). In parallel, thymol, a natural phenolic compound from thyme essential oil, serves as both an effective antimicrobial agent and a plasticizer, enhancing film flexibility (Kazemi-Pasarvi et al., 2020; Wu et al., 2014). The combined incorporation of $TiO_2$ nanoparticles and thymol into PLA matrices has not been extensively investigated and represents a promising strategy for developing multifunctional, eco-friendly active packaging systems aimed at reducing microbial spoilage, improving barrier properties, and extending the shelf life and quality of perishable products such as fresh fish.

## Materials and methods

Films were prepared by the solvent casting method using neat PLA pellets (10% $w/v_{solvent}$) dissolved in the film-forming solution (chloroform), with $TiO_2$ nanoparticles incorporated at concentrations of 0, 1.5, and 3% $w/w_{polymer}$ and dispersed by ultrasonication for 30 min. A fixed 12% $w/w_{polymer}$ concentration of thymol was added to one series of PLA-NPs films. Solutions were cast into 14 cm Petri dishes to achieve films with a thickness of approximately 100 μm.

The produced films were evaluated for mechanical (tensile strength, elastic modulus, elongation at break; ASTM D882-02), optical (color, opacity, light transmittance, UV barrier), and barrier properties (water vapor permeability, WVP; ASTM E96/E96M). Antimicrobial activity was assessed *in vitro* via disk diffusion assay against specific foodborne pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes*). Surface microstructure and chemical composition were characterized using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) (4000–400 $cm^{-1}$), respectively, while crystallinity was determined by X-Ray Diffraction (XRD). Finally, a shelf-life study was conducted on gilthead seabream (*Sparus aurata*) fillets stored at 2 °C for 11 days, to evaluate the films' preservation capacity, by monitoring Total Viable Counts (TVC), *Pseudomonas* spp. counts, and pH levels.

## Results

Thymol addition reduced tensile strength and elastic modulus but significantly increased elongation at break (%), while $TiO_2$ NPs had no notable impact on mechanical properties. Both thymol and $TiO_2$ NPs increased the total color difference, with higher $TiO_2$ concentrations also raising opacity values; thymol did not affect opacity. Films containing $TiO_2$ NPs exhibited excellent UV-Vis barrier properties, with 0% transmittance at 280 and 320 nm and less than 1% at 400 nm. Water vapor permeability (WVP) was significantly reduced by thymol, while $TiO_2$ NPs had little effect. All active films, particularly those with thymol, showed antimicrobial activity against all tested pathogens, notably inhibiting the growth of *E. coli*, *S. aureus*, and *P. aeruginosa*. Regarding the shelf-life assessment, PLA films containing 3% $TiO_2$ NPs and thymol achieved optimal results, reducing microbial counts by at least 1 log CFU/g and extending fish shelf life by 2 days.

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# THE IMPACT OF DIET AND FEEDING FREQUENCY ON THE PERFORMANCE OF JUVENILE *Gammarus* IN INTENSIVE CULTURE SYSTEMS

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## Introduction

To meet the rising demand for sustainable feed ingredients in aquaculture, new, alternative sources must be explored. Gammarids represent a promising low-trophic option due to their favorable protein and fatty acid profiles, as well as their role as a natural food source for many fish species (Harlıoğlu and Farhadi, 2018). They are also well-suited for high density cultivation and can be reared on a variety of marine and terrestrial side streams (Ribes-Navarro et al., 2022, Alberts-Hubatsch et al., 2019). For efficient production in intensive systems, it is crucial to understand how different diets and feeding strategies influence their growth and survival to be able to optimize production.

## Materials and methods

In this study, juvenile marine *Gammarus* were fed over a six-week period with one of three diets: fresh *Fucus* sp., commercial fish feed, or dried sugar kelp *Saccharina latissima*. Each diet was provided at two feeding frequencies, with feed supplied *ad libitum* once and three times weekly. The experiment was conducted in quadruplicates for each treatment, with 30 individuals assigned randomly to each beaker. The experimental units, filled with 700 mL of seawater (35 ppt, 99% DO, pH 8.1), were kept at a constant temperature of 12.3 °C and a 12L:12D light regime. Approx. 50% of the seawater was changed three times a week for all beakers.

Gammarids were sampled at the beginning and at the end of the experiment to obtain data on survival, total lengths, as well as the corresponding individual dry weight (DW) and carbon and nitrogen (CN) samples. Moreover, data on length measurements and survival was collected bi-weekly.

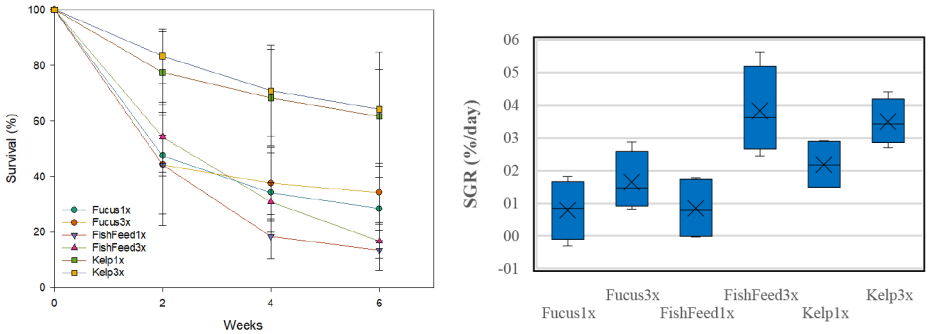


*Figure 1: Temporal survival (%, left) and specific growth rate (% per day, right) of gammarids provided three different diets over a 6-week period.*

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## Results and discussion

Preliminary results from the feeding experiment show that gammarids fed with sugar kelp had the highest survival rates across both feeding frequencies, and that the feeding interval had no significant effect on the survival of *Gammarus* across the dietary treatments. Gammarids fed with fish feed three times per week exhibited a significantly higher specific growth rate (SGR) compared to those in the Fucus treatment at the same feeding frequency. The juveniles in the fish feed treatment provided three times a week also had significantly higher SGR than the ones who were fed once a week. Otherwise, no significant differences in SGR were observed between the other diets or feeding frequencies.

## Conclusion

Overall, the diet had a stronger impact on the survival of the gammarids than the feeding frequency. A higher feeding frequency promoted higher growth, although not significant, and a further increase in feeding frequency (e.g. daily) could be explored. Fish feed fed three times a week resulted in a high growth rate, but overall poor survival. Thus, it can be concluded that the dried sugar kelp had the best effect on both growth and survival among the tested diets.

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# DEVELOPMENT USING BIOLUMINESCENCE IMAGING OF A RECOMBINANT ANGUILLID HERPESVIRUS 1 VACCINE CANDIDATE ASSOCIATED WITH NORMAL REPLICATION IN VITRO BUT ABORTIVE INFECTION IN VIVO

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## Introduction

Anguillid herpesvirus 1 (AngHV-1) (recently renamed Cyvirus anguillidallo 1) is the etiologic agent of a lethal disease that affects several eel species. It is thought to be one of the main infectious agents causing a population decline in wild eels and economic loss within the eel aquaculture sector. Since the first report of AngHV-1 in the 1980s, it has been detected globally, affecting different anguillid eel species from temperate to tropical countries. To date, there is no vaccine against AngHV-1 available on the market. Current measures to prevent AngHV-1 disease within aquaculture settings mainly rely, when possible, on shifting water temperatures outside of the range (below 22 °C) associated with efficient lytic replication. However, it promotes latent infection thereby increasing the risk of transmission when latently infected eels are cohabiting with naïve eels under stressing conditions. Recently, we developed an attenuated recombinant vaccine against CyHV-3 using prokaryotic recombination technologies and in vivo bioluminescent imaging system (IVIS). The development of this vaccine relied on the deletion of CyHV-3 ORF57. Orthologues of CyHV-3 ORF57 exist in CyHV-1 and CyHV-2 (ORF57) and in AngHV-1 (ORF 35). Therefore, this study demonstrates the effect of ORF35 deletion on the ability of AngHV-1 to replicate in cell culture and on its virulence after inoculation in European eels (yellow eel stage) by different routes.

## Materials and methods

Eel kidney (EK-1) cells were cultured. The AngHV-1 isolate (UK strain) was used as a parental strain to produce recombinant AngHV-1 UK Luc strain, UK ORF35 Del and UK Luc ORF35 Del by homologous directed recombination (HDR). Luc strain encodes a LucGFP (luciferase and copGFP) cassette inserted in the intergenic region ORF32–ORF33 of AngHV-1 genome. ORF35-deleted recombinants were produced by replacing ORF35 with a sequence encoding mCherry. All the recombinants in this study were confirmed by SacI restriction fragment length polymorphism (RFLP) and transcriptional analyses. Indirect immunofluorescence staining, viral growth and plaque-area assay for recombinant AngHV-1 strains were investigated. These recombinants were inoculated in eels by intraperitoneal injection or intradermic infection or immersion. At specific days post infection, the eels were euthanized to measure the bioluminescence radiance using IVIS for the both sides of the body, gills and internal organs. We tested the potential of different concentrations of AngHV-1 UK ORF35 Del strain as a vaccine candidate for the mass vaccination of yellow eels by immersion (primary infection) along with a group of eels exposed to psoralen/UV inactivated AngHV-1 ORF35 Del strain (highest dose) to test the hypothesis of infectivity of the virus, acting like an inactivated vaccine. At 36 days post-exposure, eels were challenged with the AngHV-1 UK Luc strain and analysed it in IVIS at specific time points.

## Results

The replication kinetics of ORF35-deleted recombinant strains (UK ORF35 Del and UK Luc ORF35 Del strains) reached high and comparable titers with no significant statistical differences detected with parental strains (UK and UK Luc strains). The UK Luc and the UK Luc ORF35 Del strains expressed both luciferase bioluminescence and copGFP fluorescence, while mCherry fluorescence correlated with ORF35 deletion. Plaque-area assays revealed that the two ORF35 Del strains produced significantly smaller plaques than the wild-type parental strains. AngHV-1 ORF35-deleted strains using this approach demonstrated that ORF35 is not essential for viral replication in cell culture. For IP inoculation in eels, UK Luc ORF35 Del strain expressing

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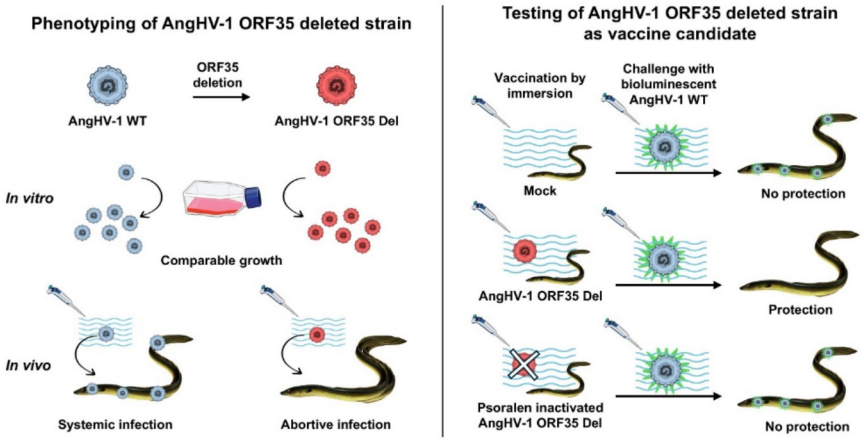


Figure: Graphical illustration of research findings

either much lower or no bioluminescence signals when compared with UK Luc strain. Similar to IP, immersion infection revealed significant differences between parental and ORF35 Del strains. While intradermal inoculation showed that the fish infected with either the UK Luc or UK Luc ORF35 Del strains showed positive signals at 2 dpi but only in the skin then these signals reduced at later time points in eels infected with UK Luc ORF35 Del. This suggested that the inoculation led to an erratic and abortive infection despite the artificial systemic mode of infection used. In the case of different concentrations of UK ORF35 Del strain as a vaccine candidate, at 6 dpi and at later timepoints, all the eels from mock and Psoralen/UV-inactivated groups expressed infection in the skin, gills and also in the other organs tested (brain, heart, and gut–liver). A significant effect of UK ORF35 Del was observed for all doses tested for the skin and the gills, while a significant effect was observed for the brain, the heart, and the gut–liver at doses of 50,000 pfu/mL or higher. It shows the ability of ORF35 Del strain to confer a protective immunity against a wild-type challenge.

**Conclusion**

The present study investigated, using recombinant strains and bioluminescent imaging, the effect of AngHV-1 ORF35 deletion on virus replication in vitro, virulence in vivo, and the potential of AngHV-1 ORF35-deleted recombinants as vaccine candidates for the mass vaccination of eels by immersion. The results suggest that the AngHV-1 ORF35 expresses singular and essential properties in vivo compared to its orthologues encoded by CyHV-2 and CyHV-3. This study supports the potential of AngHV-1 ORF35-deleted recombinants as vaccine candidates for the mass vaccination of eels by immersion.

# MITIGATION OF POST-HARVEST CONTAMINATION IN FISH BY A METHYL-CELLULOSE COATING INCORPORATING BACTERIOPHAGES TARGETING *Vibrio alginolyticus*

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**Introduction**

Post-harvest contamination poses a significant challenge in aquaculture, directly affecting the microbiological quality and shelf life of fish products. *Vibrio alginolyticus* is among the predominant microorganisms in marine fish. In the pursuit of sustainable and targeted antimicrobial interventions, bacteriophages have emerged as promising biocontrol agents. This study investigates the application of the lytic bacteriophage Athena 1, incorporated into a methyl-cellulose-based edible coating, for the control *V. alginolyticus* V1 on fresh fish. The investigation focuses on assessing the phage's stability, lytic efficacy, and antimicrobial efficacy within the coating matrix under conditions that simulate post-capture handling and storage.

**Materials and Methods**

The lytic bacteriophage Athena 1, specific to *Vibrio alginolyticus* strain V1, was evaluated for its stability across a range of pH values and temperatures to confirm its viability during coating preparation. Subsequently, Athena 1 was directly applied to surface-contaminated *Argyrosomus regius* fillets to evaluate its standalone antimicrobial activity. A phage bioactive coating (PBC) was formulated by incorporating Athena 1 (~$10^9$ PFU/mL) into a 2% (w/v) methyl-cellulose solution. The coating was applied to fish fillets by immersion, and its in vivo efficacy against *V. alginolyticus* was evaluated over a 48-hour incubation at 25°C. Microbiological analysis included enumeration of Total Viable Counts and *Vibrio* spp., while phage persistence was monitored through plaque assays and absolute quantification via RT-qPCR. Furthermore, a predictive growth model was employed to estimate the kinetic parameters of *V. alginolyticus* under each treatment, providing a deeper understanding of the inhibitory effects of the coating and the growth dynamics of the target bacterium on fish fillets.

**Results**

Athena 1 exhibited strong lytic activity under the condition required for coating preparation, demonstrating its stability and robustness. Direct application of the phage onto fish fillets resulted in a significant reduction of *Vibrio alginolyticus* V1 growth compared to untreated controls, indicating effective antimicrobial activity even withing the complex matrix of fish tissue (Figure 1).

The *in vivo* application of the phage incorporated into a methyl-cellulose-based bioactive coating (PBC) effectively delayed bacterial growth by 1.68 $\log_{10}$ CFU/mL, supporting previous findings. To further validate its performance, an accelerated shelf-life study was conducted. The growth rate of *V. alginolyticus* V1 was significantly lower in the PBC-treated group (0.395 ± 0.0791) compared to the coating without phage (0.748 ± 0.0973) and the untreated control (1.026 ± 0.163) (Figure 1). Additionally, absolute quantification of bacteriophage Athena1 during the accelerated shelf-life study demonstrated a time-dependent increase in phage levels following coating application. A statistically significant exponential rise (approximately $10^4$-fold) in PFU levels was observed within the first 8 hours post-application, followed by a plateau phase that persisted for at least 16 hours.

These findings highlight the potential of Athena 1 as an effective antimicrobial agent within methyl-cellulose-based biodegradable coatings for fish, further supporting the promising role of bacteriophages in mitigating spoilage and extending the shelf life of aquaculture products.

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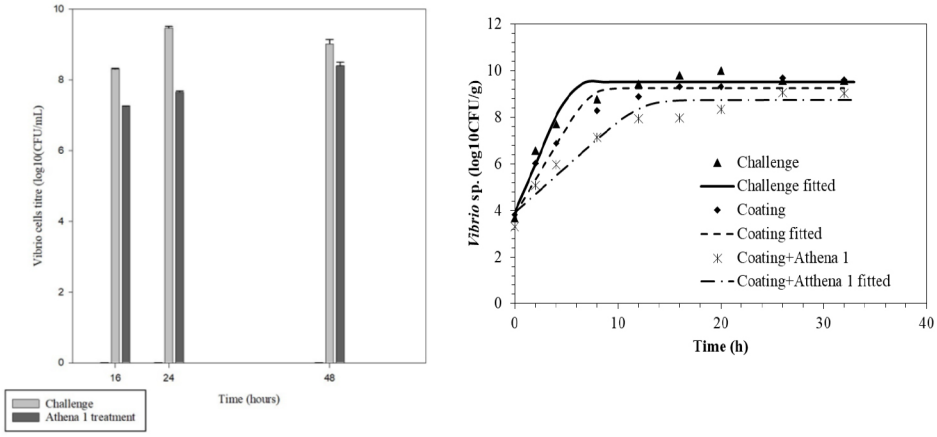


Figure 1 Left: Bacterial titre of *Vibrio* species counted on TCBS agar plates coming from challenged (Challenge) and Athena 1 treated and challenged (Athena 1 treatment) fish samples incubated at 25°C for 48 hours. Right: Growth data and the fitted Baranyi and Roberts curves of the growth of *Vibrio* cells on TCBS agar plates of challenged (Challenge), coated and challenged (Coating) and methylcellulose phage bioactive coated and challenged meagre fish fillets (Coating+Athena1) incubated at 25°C for 32 hours

# RESOLVING SPECIES-SPECIFIC FUNCTION IN MICROBIOME ANALYSIS IS KEY TO ENVIRONMENTAL MANAGEMENT DECISIONS IN AQUACULTURE

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The functional dynamics within bacterial communities are increasingly recognized as crucial to the sustainability and health of cultured aquatic species. Interest in microbiome analysis has soared in response and, consequently, a number of studies have examined the bacterial composition of land- and sea-based aquaculture sites. The majority of investigations use NGS platforms to sequence the hypervariable V3/V4 region of the bacterial 16s rRNA gene, providing identification and relative abundance data for all genera. While the V3/V4 region can also provide species-level differentiation in some cases, the necessary resolution for accurately defining closely related species or detecting uncommon or recently identified genera is lacking. Bacterial metabolism, growth strategies, and capacity for beneficial or antagonistic microbial interactions are broadly species dependent. The work presented here represents a compilation of data from our custom full-length (FL) 16S rRNA gene sequencing platform and database. We describe species-specific bacterial functions related to recirculating aquaculture system (RAS) environmental management and the monitoring of open-water sites, including: nitrification, the metabolism of eutrophication-relevant nutrients, and off-flavour chemical or cyanobacterial toxin production.

We find that full-length 16S rRNA identification of important bacterial groups such as Cyanobacteria is key to avoid overrepresenting production risk from benign species and for the selection of appropriate management methods of undesirable species. Furthermore, we have identified bacterial storage and subsequent release of nutrients as a hidden risk for water quality management and reporting in open water shellfish sites. Lastly, the prediction of biofilter establishment, maturation, function, and tolerance to change is poorly represented at a genus level, particularly amongst nitrite-oxidizing bacteria, such as Nitrospira. These examples and others will display the scientific value of full-length 16S rRNA sequencing in environmental surveillance of land and open water aquaculture sites and, therefore, critical management decisions for all aquaculture producers.

# BALANCING COMMON CARP (*Cyprinus carpio*) PRODUCTIVITY AND SUSTAINABILITY IN WATER-SCARCE POND AQUACULTURE SCENARIOS

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## Introduction

Freshwater aquaculture, particularly pond-based systems, is increasingly challenged by the growing prevalence of water scarcity, an issue compounded by climate change, population growth, and competing demands for freshwater resources. Projections indicate that climate-induced variability in precipitation, increased evapotranspiration, and declining surface water availability will disproportionately impact traditional aquaculture regions reliant on open water systems. In this context, the sustainability of pond aquaculture is under scrutiny, prompting the need for adaptive management strategies that can maintain productivity without exacerbating environmental stress. Intensification has emerged as a key strategy to increase output per unit of water, often through increased stocking densities and enhanced feeding practices. However, the transition from extensive or semi-intensive to more intensive systems introduces complex trade-offs. Higher biomass densities and nutrient inputs can lead to water quality deterioration, elevated fish stress, and greater susceptibility to diseases, particularly under water-limited conditions. This study aimed to evaluate how the interactive effects of water supply level, stocking density, and feeding regime influence growth performance, environmental parameters, and physiological stress responses in common carp (*Cyprinus carpio*) juveniles during the first production season in ponds.

## Materials and Methods

A full factorial experiment ($2^3$ design) was implemented to assess the individual and interactive effects of three key variables: Water supply: optimal (standard replenishment based on evaporative loss) vs. limited (restricted input mimicking drought conditions), Stocking density: low (standard semi-intensive density) vs. high (intensive commercial-level density), Feeding regime: traditional (plankton enhancement + cereal grains) vs. commercial (Aller Aqua formulated feeds).

Juvenile common carp (mean initial weight: 2.5 g) were stocked into twelve experimental earthen ponds (~300 m² each), with each treatment combination replicated in triplicate. The trial spanned over a three-month ongrowing period during the primary production season. Key metrics included: weight gain, specific growth rate (SGR), feed conversion ratio (FCR), survival rate, and plasma cortisol levels. Water quality: temperature, dissolved oxygen (DO), total ammonia nitrogen (TAN), and chlorophyll-a concentrations. Environmental and physiological data were collected biweekly, while growth performance metrics were recorded at the start, mid-point, and end of the trial. Statistical analysis was performed using a three-way ANOVA to detect main effects and interactions, followed by Tukey's HSD for post hoc comparisons ($\alpha = 0.05$).

## Results and Discussion

The study revealed significant main and interactive effects among the three tested variables. Water supply limitation had a consistently negative impact on fish performance across treatments, reducing both growth rate and survival. However, the extent of these effects varied significantly depending on the stocking density and feeding strategy employed.

Under limited water conditions, high-density ponds with traditional feeding showed the lowest performance in terms of growth and survival, accompanied by elevated levels of TAN and critical DO dips, especially during early morning hours. These conditions correlated with increased stress markers, including elevated cortisol levels and frequent surface gasping behavior. Conversely, the use of commercial feed under the same high-density, water-limited scenario mitigated many of these negative effects. Fish in this treatment group maintained significantly better FCR and growth rates compared to those on traditional diets, with more stable DO profiles and reduced behavioral stress indicators.

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Interestingly, under optimal water conditions, the difference between feeding regimes was less pronounced, suggesting that the benefits of formulated feed are most evident when environmental conditions are suboptimal. The commercial diet appeared to not only improve nutrient assimilation but also reduce organic loading, thereby alleviating oxygen stress and minimizing toxic metabolite accumulation.

Water quality trends supported these observations: ponds with traditional feeding showed significantly higher chlorophyll-a and TAN levels, particularly under high density and limited water supply. This suggests a synergistic effect of increased nutrient loading and poor flushing, which likely exacerbated algal blooms and microbial respiration, further lowering oxygen availability. In contrast, the commercial feed contributed less to water quality deterioration, likely due to its higher digestibility and nutrient density.

**Conclusion**

While higher stocking densities are often viewed as ecologically risky, they can be viable when aligned with high-efficiency feeding strategies and proactive water quality management. Traditional feeding approaches, though cost-effective in the short term, may exacerbate eutrophication and compromise fish welfare, particularly under drought-prone conditions.

In the context of climate change and increasing resource limitations, adaptive aquaculture practices based on factorial insights like those presented here will be essential to future-proof the sector. These results contribute to the development of more resilient freshwater production systems and inform decision-making around feed formulation, density management, and water use efficiency in the face of environmental uncertainty.

*Acknowledgments:* The study was supported by the Aquaexcel 3.0 TNA, PID26174 project.

# A GLIMPSE INTO OFFSHORE AQUACULTURE – FIRST INSIGHT INTO BLUE MUSSEL (*Mytilus edulis*), OYSTER (*Ostrea edulis*), AND ALGAE (*Saccharina latissima*) GROWTH AT THE ULTFARMS PROJECT PILOT SITE FINO3 IN THE NORTH SEA

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ULTFARMS is an EU Horizon project dedicated to unlocking the potential of sustainable offshore aquaculture by developing and testing cultivation systems for low-trophic species in co-location with offshore wind infrastructure. At the FINO3 test site in the North Sea, near the DanTysk wind farm, three systems were deployed for the cultivation of *Saccharina latissima* (kelp), *Mytilus edulis* (blue mussel), and *Ostrea edulis* (European oyster). Preliminary results demonstrate promising growth performance: mussels achieved flesh content up to 47%; kelp grew up to 1.3 mm/day despite a short cultivation period; and oysters are under continued observation (results expected by September 2025). These findings validate the technical feasibility and biological viability of offshore aquaculture and offer a foundation for further development of multi-use marine sites.

## Introduction

The European Union's blue economy strategy emphasizes climate-neutral food production, optimized marine spatial planning, and co-use of ocean infrastructure. ULTFARMS directly contributes to these goals by designing and validating aquaculture systems for low-trophic species that can coexist with offshore wind farms. The project aims to overcome technical, biological, and economic barriers to offshore farming while supporting circularity, biodiversity enhancement, and regional economic value chains, contributing to the Ocean Mission Baltic & North Sea Lighthouse. The FINO3 pilot site, located adjacent to the DanTysk wind farm in the North Sea, offers a challenging but strategic setting for testing aquaculture prototypes under real offshore conditions. The project's goal is to assess biological viability, system resilience, and scalability of co-located farming of macroalgae and filter feeders, paving the way for environmentally and economically sustainable offshore aquaculture operations.

## Materials and Methods

Three aquaculture systems were installed at the FINO3 offshore research site:
The study was conducted at the FINO3 research platform, located in the German Bight of the North Sea, adjacent to the DanTysk offshore wind farm. This location offered a high-energy offshore environment ideal for testing robust aquaculture infrastructure under real marine conditions. Within the ULTFARMS project, three cultivation systems were installed to evaluate the growth potential of *Saccharina latissima* (sugar kelp), *Mytilus edulis* (blue mussel), and *Ostrea edulis* (European oyster).

The algae cultivation system consisted of a net structure that extended from the sea surface down to a depth of two meters. It was anchored on both ends using delta flippers connected to chain links and reinforced by concrete blocks for additional stabilization. *S. latissima* specimens were sourced regionally and propagated in a laboratory setting. Two seeding approaches were employed: a direct seeding method, where spores were applied directly onto the net, and a twine-seeding method in which pre-seeded twine was attached to vertical lines flanking the net structure. The entire net system was assembled and seeded at the harbor shortly before being deployed at the offshore site. Due to unforeseen technical complications, the algae had to be harvested after only three months of growth.

The mussel cultivation system featured a submerged backbone composed of a polypropylene tube suspended approximately seven meters below the water surface. Attached to this backbone were nets. Buoyancy was provided by flotation buoys, which were evenly spaced and connected to the backbone. The structure was anchored similarly to the algae system, using delta flippers, chain, and concrete weights. Different net materials were deployed in early spring to encourage natural mussel spatfall. The system remained at sea also during winter to allow a natural grow-out phase of up to two years. Subsamples were collected periodically during routine inspection and maintenance to monitor settlement and growth progression.

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For oyster cultivation, a novel system was developed consisting of small, commercially available oyster baskets arranged within a steel cage frame. Each cage included a weighted base for stability on the seafloor. The entire unit was equipped with an acoustic remote control (ARC) retrieval mechanism mounted on the upper frame. This system could be triggered remotely, deploying a buoy attached to a retrieval line, which enabled surface access to the submerged cage. The baskets were filled with hatchery-reared European oysters verified to be free of *Bonamia* infection in order to protect the trial site from potential pathogen introduction. The oyster system was fully assembled and stocked before offshore deployment in May 2024 and shall remain on site until September 2025. Growth data is expected after the current cultivation cycle concludes.

This comprehensive setup allowed for simultaneous testing of different species, materials, and deployment strategies under the same environmental conditions, providing valuable early data for offshore multi-use aquaculture development.

**Results**

*Algae (Saccharina latissima)*
The kelp cultivation trial, despite being limited to a three-month period due to technical constraints, showed encouraging biological performance. Growth rates of up to 1.3 mm per day were observed, aligning with published benchmarks for early-stage development in offshore kelp farming. However, growth was uneven. The central and lower sections of the net exhibited moderate to good growth, while the uppermost zone showed little to no growth. This absence of biomass in the top section suggests adverse effects from high surface turbulence and wave-induced mechanical stress. No substantial difference in growth performance was observed between the direct seeding and pre-seeded twine methods.

*Mussels (Mytilus edulis)*
Mussel settlement and growth were patchy, with clear differences in performance among different tested net materials. The standard polypropelene mesh netting supported the highest spat density and showed the best results . In contrast, fuzzy rope nets had poor results. Subsamples taken during mid-season inspections and final autumn maintenance revealed well-developed mussels on the nets, with flesh content reaching up to 47%. This figure reflects excellent meat yield and is comparable to quality standards for commercial mussel production. However, variability within and between net types highlights the need for improved design.

*Oysters (Ostrea edulis)*
The oyster system is still deployed last visual inspection revealed system robustness under offshore conditions. Growth data was not available at Abstract submission time but is expected to be presented during the conference.

**Discussion**

These preliminary trials at the ULTFARMS FINO3 pilot site provide a foundational proof-of-concept for integrated, offshore aquaculture in co-location with wind energy infrastructure. Although the biological results were mixed in uniformity, each of the systems showed technical feasibility and species-specific success indicators that merit further investigation and upscaling.

The short duration of the kelp trial limited conclusions on seasonal yield, yet the observed growth rates suggest that *S. latissima* can develop efficiently in exposed sites. The absence of growth in the upper net layers likely results from physical abrasion and motion during heavy seas, indicating a need to adapt net design or focus seeding below the wave-affected zone. Future efforts should investigate longer grow-out durations.

Mussel cultivation showed high potential for biomass yield and quality. The high flesh content of up to 47% supports commercial interest, but patchy settlement highlights need for further research.

Oyster growth data remains pending. But the structural setup and ARC retrieval system look promising. This technology can drastically reduce offshore maintenance costs by removing the need for diver-based operations. Assuming acceptable growth and survival rates continue, this system may serve as a model for future offshore shellfish farming infrastructure.

These results represent a promising first step toward a scalable and environmentally compatible model for offshore aquaculture within Europe’s evolving blue economy framework.

# APPLYING A STANDARDIZED RISK-EVALUATION TO ALIEN SPECIES IN AQUACULTURE

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Aquaculture is a global industry that relies upon the culture of alien species – either referring to non-native species that were introduced through human activities or species resulting from selective breeding, hybridization or domestication that are now genetically or phenotypically distinguishable from their ancestors.

The (possible) biodiversity consequences of these species escaping into the natural environment are rarely studied, nor quantified in a consistent or standardised way. This is largely due to variability in how ecosystems respond, the delayed emergence of ecological effects, and the complexity of interacting factors that influence biodiversity. Despite this, clear cases of severe biodiversity impacts are known, e.g. crayfish introductions in Europe or (big head) carp introductions in the USA.

Policy makers, including aquaculture third-party certification schemes, are challenged with regulating the culture of alien species as considerable resources and efforts are needed to intervene in controlling these species. This has resulted in the clear need for scientific risk assessments to evaluate, compare, and predict the magnitudes of the impacts of alien taxa and to determine and prioritise appropriate measures where necessary.

The Environmental Impact Classification of Alien Taxa (EICAT) is a unified assessment method based on the mechanisms of impact used in the International Union for Conservation of Nature (IUCN) Global Invasive Species Database. EICAT evaluates alien species against thirteen potential Impact Mechanisms (e.g. competition, hybridisation) based on evidence of observed harm and classified according to five Impact Categories (Minimal, Minor, Moderate, Major, Massive). Taxa categorised in the latter three categories are considered "harmful". In addition, the Methodology also has a category for taxa that are data deficient, when no alien populations are found, or not (yet) evaluated. Based on the outcome of the Impact Mechanism and Impact Category, policymakers are better informed regarding which interventions are relevant – and justifiable towards the industry.

Here we outline the use of the EICAT methodology as part of the Aquaculture Stewardship Council (ASC) Certification Programme. We draw upon several examples of assessed species to demonstrate how scientific risk assessments can inform policy setting.

# NUTRIENT DIGESTIBILITY, FAECAL CHARACTERISTICS, AND OXYGEN CONSUMPTION IN PIKEPERCH (*Sander lucioperca*) FED UNCONVENTIONAL FEED INGREDIENTS

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## Introduction

Pikeperch (*Sander lucioperca*) is an emerging species in aquaculture, valued for its high meat quality and increasing market demand (Stejskal et al., 2023). In its natural habitat, this carnivorous species primarily consume crustaceans, insects, and fish. Under commercial farming conditions, pikeperch are typically fed formulated diets with high protein levels ranging from 43% to 50% (Policar et al., 2019). These diets often require substantial inclusion of fishmeal sourced from marine environments – a practice that raises concerns regarding long-term sustainability due to declining fish stocks and the ecological impact on marine ecosystems. In this context, alternative protein sources such as insect meal derived from yellow mealworm (*Tenebrio molitor*) and krill meal (*Euphausia superba*) have gained attention as promising ingredients for aquafeeds. These ingredients are characterized by high protein content, a well-balanced amino acid profile, and high palatability for carnivorous fish species (Tran et al., 2022; Tran et al., 2025). The present study assessed the digestibility of nutrients and energy from these alternative feed ingredients in pikeperch. The findings aim to provide insights that support the development of future feed formulations for this species.

## Materials and methods

Two ingredients, including defatted yellow mealworm (*T. molitor*) (TM), and krill meal (*E. superba*) (KM) were evaluated for nutrient digestibility. A control diet (CON) was formulated, containing 50% crude protein, 15% lipid, and 22 MJ/kg with fishmeal as a dominant protein source, in order to meet the nutrient requirements of pikeperch (Nyina-Wamwiza et al., 2005). The other two test diets contained 80% of CON, diluted by 20% with the corresponding test ingredients (TM and KM), abbreviated diets as TMD and KMD, respectively. To these diets, 0.2% yttrium oxide ($Y_2O_3$) was added as an inert marker to determine nutrient digestibility. Experimental diets were produced by a 2-mm die extruder by Research Diet Services, Netherlands. Fish were fed with the respective diets at 2% body weight for a duration of 8 weeks. During the last four weeks of the experiment, nutrient digestibility (dry matter, ash, crude protein, fat, carbohydrate, energy, phosphorus and calcium), waste production, and faeces recovery of the eight diets was determined. Moreover, oxygen consumption and ammonia production were determined from week 3 to week 7. Faeces particle size (quality) was determined from week 6 to week 8; faeces recovery was determined during week 8 after a 72-hour faeces collection.

## Results

### *Growth performance*

Dietary treatment did not significantly affect survival rate, growth indices, and feed utilization in pikeperch (Table 1).

### *Nutrient and energy digestibility*

Nutrients and energy in KM appeared to be significantly better digested by pikeperch than TM ($P < 0.05$) regarding dry matter, protein, lipid, total carbohydrate, and energy (Table 2).

### *Faecal characteristics*

There was no significant difference on faecal size distribution and recovery of pikeperch fed experimental diets (Table 3).

Collected faeces samples of pikeperch fed three diets are presented in Figure 1.

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Table 1. Survival rate and growth performance of pikeperch fed three diets for 8 weeks.

| | Experimental diets | | | SEM | ANOVA P-value |
|---|---|---|---|---|---|
| | CON | TMD | KMD | | |
| Survival (%) | 100.0 | 99.3 | 100.0 | 0.01 | 0.42 |
| Initial body weight (g/fish) | 19.2 | 19.1 | 19.0 | 0.07 | 0.15 |
| Final body weight (g/fish) | 35.1 | 33.4 | 36.2 | 0.94 | 0.19 |
| Growth (g/fish/day) | 0.28 | 0.26 | 0.31 | 0.018 | 0.19 |
| Feed intake (g DM /fish/day) | 0.30 | 0.30 | 0.29 | 0.006 | 0.36 |
| SGR (%/day) | 1.08 | 1.00 | 1.15 | 0.052 | 0.19 |
| FCR | 1.06 | 1.19 | 0.95 | 0.057 | 0.07 |

CON, control diet; TMD, diet with 20% yellow mealworm meal; KMD, diet with 20% krill meal; SEM, pooled standard error of the mean; DM, Dry Matter; SGR, Specific Growth rate; FCR, Feed Conversion Ratio.

Table 2. Digestibility (%) of feed ingredients in pikeperch (*S. lucioperca*)

| | Ingredients | | SEM | ANOVA *P-value* |
|---|---|---|---|---|
| | TM | KM | | |
| Dry matter | 76.9[b] | 97.2[a] | 3.21 | 0.01 |
| Crude protein | 90.1[b] | 98.7[a] | 1.85 | 0.03 |
| Crude fat | 94.1[b] | 101.4[a] | 0.83 | 0.01 |
| Total carbohydrate | 44.3[b] | 83.4[a] | 3.44 | 0.02 |
| Energy | 82.5[b] | 97.9[a] | 1.90 | <0.01 |

*Different letters within a row indicate significant differences (P < 0.05)*

Table 3. Particle size distribution of pikeperch faeces fed experimental diets

| | Experimental diets | | | SEM | ANOVA *P-value* |
|---|---|---|---|---|---|
| | CON | TMD | KMD | | |
| **Particle range (µm)** | | | | | |
| *>850* | 44.5 | 37.4 | 38.5 | 0.096 | 0.442 |
| *850-300* | 7.6 | 9.3 | 9.6 | 0.027 | 0.430 |
| *300-100* | 18.4 | 21.1 | 20.3 | 0.034 | 0.445 |
| *100-40* | 12.1 | 13.3 | 12.9 | 0.023 | 0.699 |
| *<40* | 17.3 | 18.8 | 18.6 | 0.026 | 0.590 |
| **Recovery (%)** | 25.5 | 36.3 | 35.4 | 4.50 | 0.25 |
| **Production (g/kg DM feed)** | | | | | |
| *Total waste production* | 188.9 | 197.7 | 157.1 | 15.08 | 0.22 |
| *Removed waste* | 47.2 | 71.5 | 55.1 | 6.84 | 0.11 |
| *Non-removed waste* | 114.7 | 125.9 | 102.0 | 15.91 | 0.28 |

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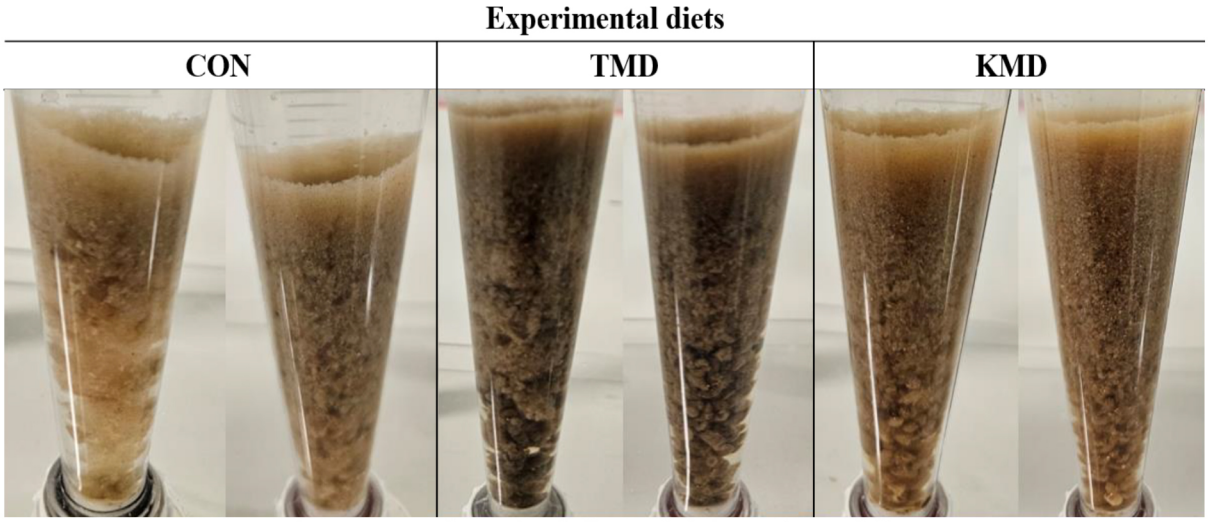


Figure 1. Faecal sample of pikeperch fed experimental diets

Table 4. Oxygen consumption of pikeperch fed three experimental diets

| | Experimental diets | | | SEM | ANOVA P-value |
|---|---|---|---|---|---|
| | CON | KM | YMW | | |
| Oxygen consumption (mg $O_2$/kg$^{0.8}$/min) | 1.61 | 1.60 | 1.65 | 0.02 | 0.54 |
| Oxygen consumed/DM feed (g/kg) | 384.60 | 383.13 | 373.92 | 4.04 | 0.55 |
| Oxygen consumed/Digestible Energy (mg/kJ) | 20.41 | 19.92 | 19.34 | 0.23 | 0.14 |

***Oxygen consumption***

There was no significant difference in oxygen consumption of pikeperch fed experimental diets (Table 4).

## Conclusion

Inclusion of 20% unconventional protein sources in the diet did not significantly influence the survival rate, growth performance, or feed utilization of pikeperch (*S. lucioperca*), indicating that this species can accept such protein sources without adverse effects on performance or palatability. Digestibility assessments demonstrated that nutrients and energy derived from krill meal are efficiently utilized by pikeperch, highlighting its potential as a dietary protein source. However, the use of mealworms should be approached with caution due to their relatively low protein digestibility. The inclusion of both mealworm and krill meal did not result in significant differences in faecal characteristics or oxygen consumption when compared to the control diets. These findings have important implications for the commercial aquaculture of pikeperch, particularly in the context of future feed formulation, as well as the management of waste collection and oxygen consumption in recirculating aquaculture systems.

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# FROM WASTE TO VALUE: INVESTIGATING MUSHROOM STEMS FROM *Pleurotus ostreatus* GROWN ON MEALWORM FRASS AS A NUTRITIONAL SOURCE FOR AQUACULTURE FEED

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## Introduction

The SAFE (SmartAqua4FuturE) project aims to revitalise EU freshwater aquaculture through a circular economy model that cuts emissions, protects biodiversity, supports sustainable feed, improves profitability, and generates jobs. It focuses on converting waste from recirculating (RAS) and integrating multi-trophic aquaculture (IMTA) into valuable resources: dried sludge for mushroom cultivation, liquid effluents for microalgae, duckweed, and watercress, which feed mealworms and redworms. These biomasses enrich feeds for European freshwater species. This study examines mealworm frass as a substrate for *Pleurotus ostreatus*, producing both edible mushrooms and protein-rich stems for aquaculture feed.

## Materials and Methods

Substrates containing 0–15% mealworm frass were prepared for *P. ostreatus* cultivation. Fruiting bodies and stems were harvested separately. Chemical analyses included determination of crude protein, amino acid profile, and fatty acid composition, with emphasis on polyunsaturated fatty acids (PUFAs). β-glucan content was quantified, and antimicrobial activity of dichloromethane extracts from stems was tested against *Tenacibaculum maritimum*, a major bacterial pathogen in aquaculture.

## Results

Increasing frass inclusion significantly enhanced the protein content of both mushroom stems (from 7.78% to 22.31%) and fruiting bodies (from 24.74% to 30.99%), with enrichment in essential amino acids at 12.5–15% frass levels. PUFAs were predominant in both tissues across treatments. β-glucan content decreased with higher frass inclusion. Antimicrobial assays revealed that dichloromethane extracts from stems cultivated on 12.5–15% frass inhibited *T. maritimum* growth.

## Conclusion

Mealworm frass is a promising sustainable substrate for *P. ostreatus* cultivation, enhancing nutritional and functional properties of mushrooms while providing protein-rich biomass for aquaculture feed. This approach supports environmental and economic sustainability in EU freshwater aquaculture by closing nutrient loops and generating value-added products from waste streams.

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Hilali *et al.,* From Waste to Value: Investigating Mushroom Stems from *Pleurotus ostreatus* Grown on Mealworm Frass as a Nutritional Source for Aquaculture Feed. Sustainability 2025, 17, 6496. https://doi.org/10.3390/su17146496

# NEITHER MATURE NOR NEW BIOCARRIERS INFLUENCE THE ESTABLISHMENT OR PERSISTENCE OF YERSINIA RUCKERI

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## Introduction

The biological removal of nitrogen compounds such as ammonium and nitrite in recirculating aquaculture systems (RAS) is primarily facilitated by nitrifying bacteria residing within the biofilter. This structure is often referred to as an "ecosystem within an ecosystem" due to the diverse microbial assemblages it supports—each with distinct metabolic and physiological traits—which collectively convert toxic ammonium excreted by fish into the less harmful nitrate through a two-step nitrification process. Monitoring studies have revealed that the biofilter represents a unique microbial niche, characterized by a notably low abundance of pathogen-related species, such as *Moritella viscosa, Psychrobacter immobilis,* and *Aeromonas salmonicida*. One plausible hypothesis is that the resident microbial communities exert inhibitory effects on the colonization of these pathogens, thereby maintaining a protective microbiological environment within the biofilter. However, the mechanisms governing pathogen establishment and the influence of various environmental and operational factors on this process remain largely unexplored. It is further hypothesized that the introduction of pathogens into the biofilter—potentially triggered by disease outbreaks or microbial imbalances in the RAS—could disrupt the native microbial community. Such disturbances may negatively impact both the diversity and functional performance of the biofilter, thereby impairing nitrification efficiency and leading to suboptimal water quality characterized by elevated levels of toxic ammonium and nitrite. Additionally, many mechanical components within RAS are not easily accessible, often leading to the generalized assumption that uniform cleaning and disinfection protocols are effective across the system. In reality, spatial heterogeneity can significantly influence the efficacy of these disinfection practices. A deeper understanding of pathogen establishment pathways is therefore essential for the development of targeted and effective disinfection strategies.

## Material and methods

A 30-day biofilter experiment was conducted under controlled environmental conditions at Nofima Tromsø, with the system maintained at 12 °C and a salinity of 12 ppt within an environmental chamber. The study was designed to evaluate the effects of *Yersinia ruckeri* introduction on biofilter function, using three treatments as part of an infection challenge. The first treatment involved waterborne exposure to Y. ruckeri at a defined concentration (XY concentration). The second treatment consisted of biomedia inoculation, wherein biofilter media were submerged for 24 hours in a Y. ruckeri suspension, subsequently rinsed with phosphate-buffered saline (PBS), and transferred to the experimental culture bottles. A preliminary laboratory trial confirmed that Y. ruckeri successfully adheres to autoclaved biocarriers after 24 hours of exposure. A third group, serving as a control, remained unexposed to Y. ruckeri. Nine 1-liter Schott bottles were used for the experimental setup, each filled with a mix of 85% mature biomedia and 15% autoclaved biomedia. Continuous aeration was provided via air stones connected to membrane pumps. Every three days, total ammonia nitrogen (TAN) levels were elevated to 4 mg $L^{-1}$ to simulate nitrogenous waste input, and removal efficiency was assessed after six hours. During the first two weeks, biomedia samples were washed with PBS and marine broth, then plated on Y. ruckeri-selective agar to quantify colony-forming units (CFUs). Concurrently, microbial communities present in the water phase were analyzed by filtering the culture water and extracting microbial DNA for further analysis. At the conclusion of the 30-day experiment, a pH-shift disinfection protocol was applied to evaluate whether disinfected biomedia could act as a reservoir for Y. ruckeri, thereby ensuring that desinfection measures effectively eliminate any remaining pathogenic bacteria.

## Results

Yersinia ruckeri was confirmed to attach to autoclaved biomedia within 24 hours of exposure. Among the experimental groups, the highest denitrification performance was observed in the treatment where the inoculated biomedia were washed with PBS buffer prior to their introduction into the system. Over the course of the experiment, a gradual decline in colony-forming units (CFUs) of Y. ruckeri was observed, indicating a reduction in the presence of the pathogen over time.

## Discussion

The results indicate that Yersinia ruckeri does not actively proliferate within the biofilm of the biomedia but can still be detected more than one week after a single infection event. While the presence of Y. ruckeri had no measurable impact on the denitrification performance of the biofilter, the PBS-washing of biomedia prior to use significantly enhanced nitrogen removal efficiency. This improvement may be attributed to the removal of older or inactive biofilm layers, thereby promoting more effective colonization by active nitrifying communities. Results from the disinfection phase of the experiment are still under analysis at the time of writing.

# VIBRIOS IN BIVALVES AND CRUSTACEANS – A SURVEILLANCE GAP FOR SEAFOOD SAFETY?

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## Introduction

Bacteria in the genus *Vibrio* are naturally found in marine and brackish waters and represent one of the main challenges to microbial seafood safety, where four species are of particular concern to human health; *V. cholera*, *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus*. However, no regulations currently address the threat posed by human pathogenic vibrios. This is of particular concern for seafood products that are typically consumed without heat-treatment, such as oysters, where the presence of vibrios can lead to gastrointestinal infections of varying severity. Products meant to be heat-treated may also be an issue, as bacteria on these products can contaminate other food-items during preparation. In addition, products imported from countries with frequent use of antibacterial agents may contain resistant bacteria and contribute to spreading antibiotic resistance.

## Materials and Methods

In 2021-2024, 96 samples of fresh or frozen bivalves and crustaceans were analysed for the presence of vibrios as part of a monitoring programme by the Norwegian Food Safety Authority (NFSA) for veterinary control on seafood products imported to Norway from countries outside EU/EEA [1–5]. In addition, in 2025, 32 samples of fresh bivalves collected along the Norwegian coast, were analysed. Samples were analysed according to the NMKL method no. 156. Further, two isolates of *V. parahaemolyticus* from imported shrimp were phenotypically tested for antibiotic resistance, genome sequenced and analysed for the presence of genes associated with antibiotic resistance.

## Results

From the imported seafood samples, 12 of 96 samples contained one or more of the four target vibrio-species[1–5]. Of these, five consisted of fresh oysters, whereas the remaining seven vibrio-positive products consisted of frozen shrimp and oysters. Oyster samples were positive for *V. alginolyticus*, whereas shrimp samples were positive for *V. parahaemolyticus* or *V. cholera*. Two *V. parahaemolyticus* isolates examined further expressed phenotypic resistance and harboured resistance genes against clinically important antibiotics, including antibiotics banned in aquaculture. For the samples of Norwegian produced bivalves, 30 of 32 were positive for *Vibrio* spp., with preliminary analyses indicating the presence of *V. alginolyticus* and *V. parahamolyticus*. The two *V. parahamolyticus* strains tested further expressed phenotypic resistance, and harboured resistance genes against several clinically important antibiotics[6].

## Conclusion

These findings highlight the need to strengthen the surveillance and regulatory framework for pathogenic *Vibrio* spp. in seafood.

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# EARLY REARING CONDITIONS AND THEIR INFLUENCE ON ATLANTIC SALMON (*Salmo salar*) PERFORMANCE LATER IN LIFE

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## Introduction

Atlantic salmon smolt production in Norway has been intensified over the past years and has led to concern about increasing prevalence of life-style diseases in cultured Atlantic salmon (Frisk et al. 2021). Temperature and water salinity are critical rearing parameters that differ between smolt hatcheries (Kristensen et al. 2011) and directly affect Atlantic salmon physiology. While several studies have examined these factors in short-term experiments (Handeland et al. 1998, Handeland et al. 2000), there is limited documentation on how smolt rearing history changes long-term growth, welfare, and slaughter quality. In this study, we evaluated the short- and long-term effects of four temperature groups: 8°C, 12°C, 14°C, and a group with temperature increased from 8°C to 12°C. We hypothesized that lower rearing temperature in fresh water would result in better growth at sea. In addition, we evaluated the potential benefits of acclimatizing smolts to salinity prior to seawater transfer. All treatments were assessed in both flow-through (FT) and RAS systems.

## Material and Methods

The experiment consisted of a smolt production phase, when treatment groups were established, followed by seawater grow-out until slaughter (Fig. 1). The first phase of the experiment was conducted simultaneously in flow-through and RAS systems at Nofima's Research Station for Aquaculture in Sunndalsøra, Norway. A total of 4800 parr (10 g) were distributed across 32 rearing units and pit-tagged after reaching 20 g. All fish were exposed to a photoperiodic regime consisting of over 5 weeks of 12 hours of light and 12 hours of darkness, followed by 350 degree-days of continuous 24-hour light to induce smoltification. After smoltification, water salinity was either increased directly to 32 ppt (A), or gradually over a three-week period (B). Thereafter, 2560 smolts were and stocked in net pens at a seawater facility for grow-out. During the seawater period, the fish were kept in a common garden design. Fish and water samples were collected multiple times throughout phase 1, and at the end of the seawater phase. The analysis of response variables included water quality characterization, growth, survival, and several morphological and lab-based welfare indicators (Noble et al. 2018).

## Results and discussion

Throughout the experiment, the fish from the different treatments had low mortality and a seemingly good welfare status. During the freshwater phase, all groups developed typical smolt characteristics, indicated by an increased Na+, K+-ATPase (NKA) activity in gills, kidney, and intestine in response to 24 hours light underlining the importance of photoperiod for smolt development, irrespective of temperature. Even though the NKA development was similar in all groups, overall lower levels of NKA activity in fish held at 8°C and a less pronounced reduction in condition factor, suggest that these fish may have needed more time than the 350 degree-days to complete smoltification. Yet, serum analyses after seawater challenge tests before seawater transfer showed good and similar hypoosmoregulatory ability in all groups. Interestingly, there were distinct differences in water quality between FT and RAS systems, with an accumulation of major cations and $CaCO_3$ seen in RAS, irrespective of temperature regime, over time. This finding can potentially explain differences seen in kidney NKA activity between FT and RAS.

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Surprisingly, salmon from the 12°C and 14°C had a significantly higher thermal growth coefficient, and larger final body mass (4.4 *vs* 3.3 kg) than their conspecifics from the 8°C and 8-12°C groups. Also, the slaughter quality score was higher in these groups. Yet, there was a tendency for smaller hearts in the fish of the 12°C and 14°C groups at the end of the experiment, and it can be speculated if this could have compromised these fish in case of a disease outbreak or during strong stress exposure, however, no disease outbreak occurred, and all groups handled exposure to lice treatments equally well. During the seawater phase the fish experienced almost ideal growth conditions, with low stress and high-quality feed. Under favorable conditions, early rearing temperatures up to 14°C lead to faster production of high-quality salmon for consumption. However, potential effects on robustness should be investigated in future studies.

**Ethical statement on animal welfare**

The experiment was conducted following the EU legislation on animals in science and was approved by the Norwegian Food Safety Authority (ID#29982).

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# TESTED OFFSHORE NATURE INCLUSIVE DESIGN AS A NATURE RESTORATION MEASURE BASED ON A FILTER APPLICATION CONCEPT SHOWCASING THE POTENTIAL VALORISATION OF EXISTING AND PLANNED OFFSHORE INFRASTRUCTURE

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**Introduction:**
The biodiversity crises, habitat degradation along with a rapidly increase use of marine areas are major challenges worldwide and especially within the EU. The associated start of implementation of the EU Nature restoration regulation in aquatic environments fuels the discussion on suitable measures. These urgent requirements and rapidly progressing demands also affect aquaculture operations and cannot ignored by them. Offshore multi-use projects are currently developing concepts and testing different solutions to find and push useful measures. Nature inclusive design (NID) and artificial reefs (AR), have raised significant attention, as being a potential valuable marine ecosystem management tool, compensating human induced habitat destruction and its consequences for marine wildlife. However, a positive impact for the environment, can only be ascertained to very few installations. Missing concepts, insufficient data of sound experimental design, quantifiable objectives, monitoring strategy and data accessibility or analysis are the main reasons why the vast AR/NID-installations are not reliable to demonstrate an impact due to recent metadata analysis studies (Paxton et al., 2020). While this research gap has been increasingly acknowledged in recent years, still only a few approaches addressed the missing points for an objectively successful AR/NID-installation. Due to the ongoing expansion of marine infrastructure and offshore wind industry the pressure on habitat situation is growing but also new options arise for potentially novel installation opportunities and multi-use efforts. Within the offshore multi-use project ULTFARMS a concept with a filter-based approach has been developed and applied based on proven installations to conceptualize a species- and site-specific AR/NID-installation. The results of first tests in offshore conditions provided invaluable technical insights for future upscaled scenarios. A novel, in-house developed attachment technique, enables a non-disruptive, flexible positioning of add-ons for both existing and future offshore infrastructure, such as monopiles.

**Methodology:**
The filter approach concept builds on three sequential steps, containing multiple interrelated aspects (non-hierarchical) found in literature (see figure 1)

1. Background analysis:
The ULTFARMS Baltic Sea German offshore pilot, the research platform has been investigated before the design phase to deliver the baseline for habitat developments, cumulative human pressures, historic record of past species abundances and the current status of populations. To be an effective management tool, NID installations must employ SMART objectives prior to the installation as specific, measurable, achievable, realistic and time-bound (Higgins et al. 2022). The dramatic decline of fish populations has been identified as one of the most obvious negative changes for this area. Therefore, this restoration measure focuses to provide lost habitat for juvenile fish.

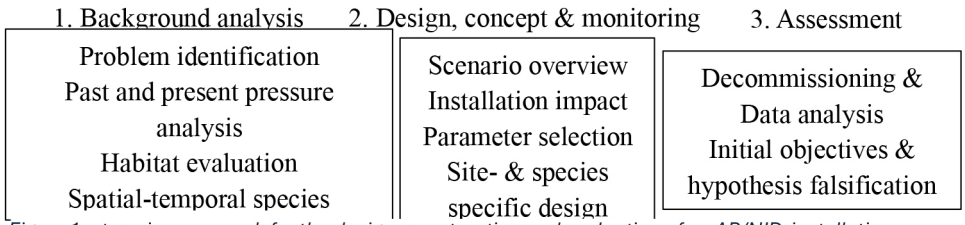

*Figure 1: stepwise approach for the design, construction and evaluation of an AR/NID-installation.*



*Figure 1: Nature inclusive design (NID) installation equipped with species-specific materials attached to the monopile of FINO2, Baltic Sea, Germany*

2. Design, concept & monitoring

A total of 42 studies have been selected, to fine-tune the site- and species-specific design. While there are limitations in predictability recent studies found good correlation between specific design requirements and population development. Fish diversity has proven to be enhanced via the provision of three-dimensional habitat that incorporates a wide range of microhabitat sizes and types (Taira et al., 2024).

3. Assessment

Stationary underwater cameras fulfil a range of requirement for offshore monitoring as very low maintenance schedules, automatic self-cleaning, continuous data over a longer period of time for monitoring fish and options for AI based automatic species identification.

**Results & discussion:**

Throughout this pilot-scale deployment, substantial amounts in both quantity and quality of fish assemblages have been observed in and around the installation. The data show a first proof of effectiveness of this NID-installation, the technical and logistical feasibility of a novel attachment technique within an existing windpark. Future experimentally sound studies shall incorporate the above-mentioned filter approach to implement, site- and species-specific AR/NID-installations.

Based on these transferable results of a modular, flexible and scalable NID design tested on an existing offshore monopile future upscaled scenarios for numerous offshore windparks are feasible.

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# THE IMPACT OF REARING ENVIRONMENT ON LARVICULTURE IN PIKEPERCH (SANDER LUCIOPERCA) UNDER COMMERCIAL CONDITIONS – IMPROVEMENTS BY MODIFYING PHOTOPERIOD, ARTEMIA ENRICHMENT, AND TEMPERATURE

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## Introduction

Intensive pikeperch larviculture presents a challenging task and is still the limiting factor for the expansion of successful production. In addition to a high level of cannibalism, which causes high mortality rates, this is primarily due to the quality of the fingerlings produced. The loss of quality is significantly reflected in the deformation rate, which in turn increases cannibalistic behavior. In pikeperch, it is known that this phenomenon mainly occurs between days 19 and 39 post-hatch, depending on the rearing temperature, which is a rather long period from a production point of view. In addition to an existing dark period, cannibalism is also promoted by the habituation to commercial dry feed, as the nutrient composition changes drastically and the prey stimulus changes due to a lack of agility. However, the drastic change in the nutrient composition, especially if no appropriate biosynthesis of enzymes has taken place, caused by live food reduction, can lead to nutritional deficiencies and consequently to deformities. Nevertheless, the rearing temperature is a success-determining factor in addition to those already mentioned being decisive for the rate and quality of metabolism, but can also detriment development. Overall, it is evident that all effects have common factors and interact among each other.

## Material & Methods

Besides the effect of two different photo regimes (16/L:8/D vs 24/L:0/D), two different rearing temperatures (16 °C vs 19 °C) were examined in two identical recirculated systems. Simultaneously, we compared the influence of the dietary enrichment of *Artemia* (enriched vs non-enriched). The trial was conducted in a multi-factorial design and groups were duplicated (model: $2^{\wedge 3}$; 8 combinations; 16 tanks). During 47 days, multiple variables were followed: growth parameters - body weight (g) and length (mm) and their coefficients of variation, condition factor and specific growth rate. In addition, the occurrence of jaw and spine deformities, survival and swim bladder inflation dynamics were assessed. Main effects and interactions were analyzed with SPSS 29.0 (IBM Co., Armonk, NY, USA) in the General Linear Model, Univariate ANOVA. Afterwards, Principal Component Analysis and Pearson correlation (n-1) were applied to compare the strength of factors. (XLSTAT, Long Island: New York, NY, USA, 2023).

## Results

Our findings revealed that a higher temperature had significant positive effect on the speed and efficacy of swim bladder inflation at day 12 ($p = 0.003$), day 23 ($p = 0.002$), and 47 ($p < 0.001$) post hatch, as well as on the total length ($p < 0.001$) and wet weight ($p < 0.01$) on day 47 post hatch. Furthermore, a higher rearing temperature improved homogeneity in growth, visible by a lower coefficient of variation (CV wet weight: $p = 0.014$). Considering photoperiod, it became apparent that it positively influenced growth shown in a higher wet weight ($p = 0.037$) at final sampling time. The third factor investigated, the enrichment of the *Artemia*, had a significant effect on the survival rate ($p = 0.005$). These results indicate that rearing pikeperch larvae at a relatively higher temperature of 19 ºC could improve the rearing success, while the enrichment of *Artemia* nauplii seems necessary. To reach a stable juvenile production, further investigations are required to determine the most efficient conditions with all crucial parameters e.g. a practical photo regime, algae product and its application rate (i.e. turbidity level), as well as applying older stages of *Artemia* (i.e. Instar II) aiming for reliable, efficient and universally applicable protocol.

# POPULATION DYNAMICS OF *Auchenoglanis occidentalis* FROM DADIN-KOWA DAM, GOMBE STATE, NIGERIA

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Fish and its resources has been an integral component of food security in many countries across the world, this is not limited to only coastal areas but also encompasses inland water bodies (Ahamed, Akter, Hasan, & Chakma, 2024). Human nutritional requirements depends also largely upon the resources obtained from fish such as lipids, high quality proteins (Nazeef, Ja'afaru, Ka, & Kabiru, 2021), and numerous minerals such as iodine and phosphorous (Ahamed *et al*., 2024).

The population dynamic parameters of *Auchenoglanis occidentalis* from Dadin-Kowa reservoir were studied. Population dynamic parameters such as growth, mortality and recruitment patterns were analyzed using length frequency data over 12-month period employing FiSAT II software. Findings revealed that **LWR** (***b*** constant) = 2.88, **K** = 0.72 $^{-yr}$., **L∞** = 40.91 cm and $\mathbf{T_{max}}$ = 3.57 years and **Θ' =** 3.14.

Mortality indices revealed that natural mortality (**M** = 1.39), fishing mortality (**F** = 0.22) and exploitation ratio (**E** = 0.14), **Lc/L∞ =** 0.48, **Emax** = 0.64, while **Lopt** = 26.4 cm. Uni-modal recruitment peak observed with **Lm** = 27.3 cm. Restocking program is suitable to ensure its continuous existence as it seems to have low population.

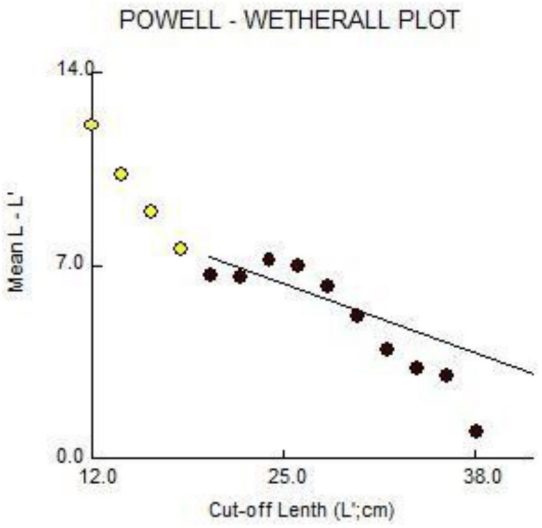


**Figure 1:** Powell Wetherall Plot of *A. occidentalis*

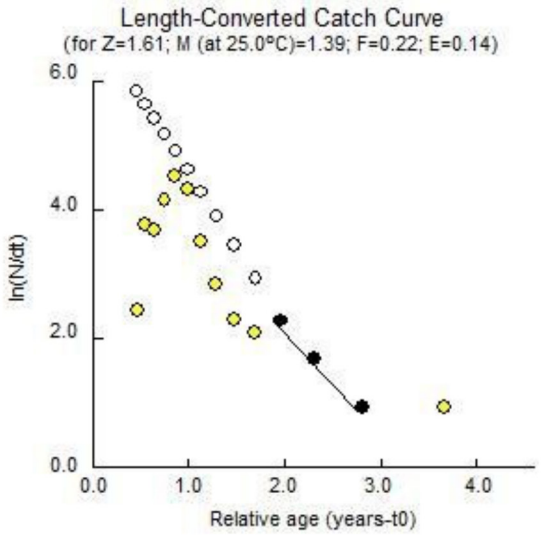


**Figure 2:** Mortality parameters of *A. occidentalis*

# QUANTIFYING THE IMPACT OF SUBOPTIMUM TEMPERATURE AND LOW DISSOLVED OXYGEN ON THE PRODUCTION BIOLOGY OF POST-SMOLT ATLANTIC SALMON *Salmo salar* USING PROTEOMICS

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**Introduction**

Temperature is the master abiotic factor for salmon growth. Atlantic salmon has a wide thermal range for growth, ranging from 12-18°C. Tasmania, which produces the majority of Atlantic salmon in Australia, is a global hotspot for ocean warming, and water temperatures exceed 18°C for extended periods during marine heat waves, which are predicted to become more frequent. Suboptimum water temperature and low dissolved oxygen (DO) are key drivers of reduced feed intake and growth during summer, yet their impact on salmon production biology remains poorly understood at the proteome level. This study used proteomics to investigate the cumulative impact of sub-optimum temperature and low DO on the growth performance and nutritional physiology of post-smolt Atlantic salmon.

**Materials and Methods**

A total of n = 1144 post-smolts with a mean weight of 419.4 ± 3.2 g were distributed across 12 indoor RAS tanks and were exposed to simulated summer conditions (19°C; 80% DO) followed by an autumn recovery (15°C; 100% DO) for 84 and 42 days, respectively. Liver and white muscle tissue samples were collected from targeted individuals for label-free quantitative proteomics (from known genotypic groups of the Tasmanian Selective Breeding Program) expected to show high, medium and low growth performance (n = 8 samples per group per time point; n = 24 per tissue per time point) after summer stress and autumn recovery, referred to as post-thermal and post-recovery time points, respectively.

**Results**

Proteomic analysis of liver and white muscle protein extracts identified ~6300 and ~1800 protein groups, respectively, and t-test analysis (FDR < 0.05) identified 3650 and 257 differentially abundant proteins (DAPs) between different time-points. Bioinformatic analysis of the liver revealed that proteins increased in the post-thermal group were associated with functional terms, including endoplasmic reticulum stress, proteasome activity, heat-shock response, antioxidant defence, amino acid catabolism, fatty acid degradation, carbohydrate metabolism, and oxidative phosphorylation Significant proteins included SERPINh1, previously identified as a key biomarker of thermal stress in Atlantic salmon (Mendoza-Porras et al., 2024). In contrast, functional terms associated with the proteins increased in the post-recovery group included ribosome biogenesis, cholesterol, and unsaturated fatty acid biosynthesis. In white muscle, enriched terms were directly or indirectly related to protein turnover processes at both time points. Statistical analysis for high vs low performance groups identified 306 and 273 DAPs in the liver between timepoints, respectively, and 81 DAPs ($p < 0.05$) at both time points in the white muscle. Enrichment analysis revealed differential mechanisms across the groups tested in this experiment.

**Conclusion**

Overall, this study provides an improved understanding of the physiological mechanisms operating in Atlantic salmon under suboptimum environmental conditions, with protein turnover being the most differentially affected process across tissues. Our findings also indicate that post-smolt salmon have biological capacity to recover and achieve compensatory growth at the protein level. Additionally, proteomic signatures across different genotypic groups were identified, offering a scope for future research towards climate-proofing the selectively bred Tasmanian salmon.

**References**

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# BENCHMARKING ATLANTIC SALMON *Salmo salar* PERFORMANCE TO TEST TRANSLATION OF RESEARCH FROM EXPERIMENTAL TO COMMERCIAL SYSTEMS USING PROTEOMICS

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## Introduction

Temperature regulates all aspects of growth and physio-metabolic processes in Atlantic salmon. In Tasmania, the production of Atlantic salmon is highly exposed to the effects of climate change due to the increasing prevalence of marine heatwaves. However, further research on the accuracy of experiments in controlled conditions in predicting farmed fish response to natural conditions is highly recommended. In this study, the cumulative effects of suboptimum environmental conditions on large salmon production biology were investigated using proteomics, and by comparing two genetically equivalent populations with similar size: one grown in an experimental recirculating aquaculture system (RAS) and the other in a commercial pen.

## Materials and Methods

In the RAS, n = 631 large salmon (mean weight ~2.5 kg) were exposed to a simulated summer of 19°C and 80% DO for 84 days, followed by 40 days of autumn recovery at 15°C and 100% DO, referred to as post-thermal and post-recovery time points, respectively. The liver and white muscle tissue samples were collected from targeted individuals (from known genotypic groups of the Tasmanian Selective Breeding Program) expected to show high, medium and low growth performance (n = 8 samples per group per time point; n = 24 per tissue per time point). In addition, this study collected same type and number of tissues (n = 24) from genetically equivalent individuals from a commercial pen at the end of a natural summer (~16.75°C; 78.92 % DO) to compare the proteomic response with the RAS simulated summer.

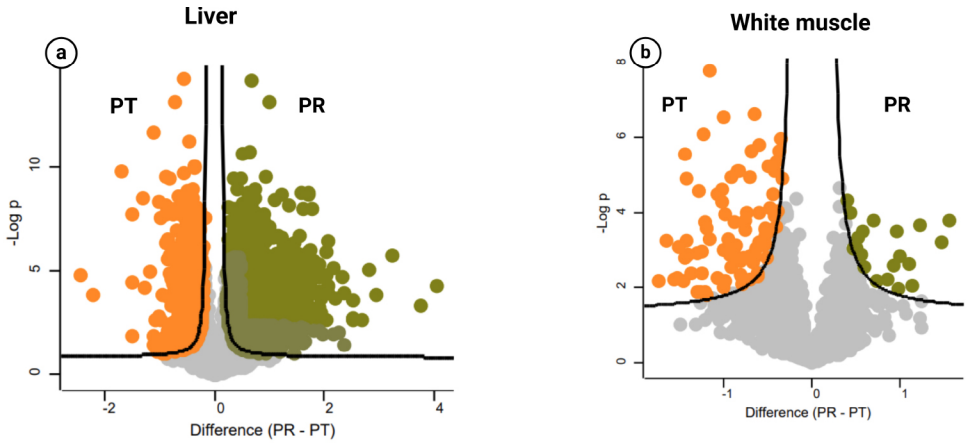


Figure 1: Volcano plots displaying the differentially abundant proteins (DAP) expressed in liver and white muscle at PT vs. PR, using FDR < 0.05 and S0 = 0.1. PT; Post-thermal PR; Post recovery.

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**Results**

In the RAS, proteomic analysis of the liver and white muscle identified ~6900 and ~1900 protein groups, respectively. Statistical analysis (FDR < 0.05) of the liver and white muscle proteomics data identified 2614 and 119 differentially abundant proteins (DAPs), respectively, between the two timepoints (Figure 1). When comparing simulated with natural summer, 3983 and 357 DAPs distinguished RAS from the pen. In the RAS, gene ontology analysis of the liver identified terms related to protein folding, SERPINh-1, and lactate metabolism among the proteins increased in the post-thermal group, and lysosomes, ribosome biogenesis, and unsaturated fatty acid biosynthesis in post-recovery. In white muscle, glucose and pyruvate metabolism, were enriched in both timepoints, with protein folding enriched only at post-thermal. In the pen, gene ontology analysis revealed shared cardinal markers (SERPINh-1), and mechanisms (endoplasmic processing, protein turnover) related to thermal stress with the RAS, while immunomodulatory proteins of innate and humoral responses were enriched only in the pen, capturing the different nature of commercial environment.

**Conclusion**

Overall, the results showed increased energy reallocation during suboptimum environmental stress and prolonged physiological recovery in large Atlantic salmon. Additionally, the findings support the use of an experimental RAS as a potential translational tool for commercial applications.

# STUNNING NEWS: EFFECTS OF IN-WATER ELECTRICAL EXPPOSURE ON BRAIN FUNCTION IN NILE TILAPIA (*Oreochromis niloticus*) AND AFRICAN SHARPTOOTH CATFISH (*Clarias gariepinus*)

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**Background**

Nile tilapia (*Oreochromis niloticus*) and African sharptooth catfish (*Clarias gariepinus*) are two important aquaculture species whose global production have increased significantly over the last decades (FAO, 2022). Ensuring a humane end of life for these species is essential for a number of interconnected reasons, spanning ethical, legal, scientific, and societal considerations. Common slaughter methods for these fish, such as asphyxiation, gill cutting, decapitation and/or evisceration, fail to meet the standards of humane slaughter, which require the use of an effective stunning method prior to killing (EFSA, 2004; Lines and Spence, 2011; Robb and Kestin, 2002; WOAH, 2019). Electrical stunning has been proposed as a more humane alternative (WOAH, 2019) and species-specific laboratory studies have demonstrated that if enough electrical energy is delivered it can induce a loss of sensibility for at least one minute in both tilapia and catfish. Further, when combined with throat cutting and immersion in ice slurry, no signs of recovery were observed for either species during a 30 min post-stunning period (Brijs et al., 2021; Sundell et al., 2024). While these findings highlight a potential of electrical stunning, the effectiveness and feasibility at scale warrants further validation under commercial conditions. Therefore, we designed a study to assess the effectiveness of in-water electrical stunning of these commercially important species at the farm level.

**Material and methods**

In-water electrical stunning was tested on tilapia and catfish at a commercial RAS farm in Skåne, Sweden, using a custom-built electrical stunner (Ace Aquatec Ltd., UK; AC source, 50 Hz). Fish were captured and subjected to an electrical stunning protocol under controlled conditions (785 ± 5 $\mu$S cm$^{-1}$, 21 ± 0.3 °C), after which they were instrumented with electroencephalography (EEG) electrodes to measure brain activity for 10 min. Stunning effectiveness was assessed via the presence or absence of visually evoked responses (VERs) in the EEG. Four stunning protocols, with regards to electrode configuration and electric intensity, were tested: (*i*) head-to-tail (HT) standard intensity, (*ii*) side-to-side (SS) standard intensity, (*iii*) SS high intensity, and (*iv*) SS high intensity combined with exsanguination and ice slurry immersion. The stun duration was 40 s for catfish and 30 s for tilapia. Low intensity was 11.63 ± 0.05 V cm$^{-1}$ (0.979 ± 0.025 A dm$^{-2}$) and 10.10 ± 0.04 V cm$^{-1}$ (0.870 ± 0.029 A dm$^{-2}$) for catfish and tilapia, respectively, while high intensity was 17.48 ± 0.04 V cm$^{-1}$ (1.696 ± 0.038 A dm$^{-2}$) and 13.52 ± 0.04 V cm$^{-1}$ (1.135 ± 0.014 A dm$^{-2}$) for catfish and tilapia, respectively.

**Results**

A Kaplan meier analysis of time until recovery of sensibility post-stunning revealed no significant differences between treatment groups for tilapia ($\chi^2(3)=3.856$, P=0.277) but a significant effect for catfish ($\chi^2(3)=41.586$, P=<0.001; Figure 1). In catfish, 100 % insensibility was achieved in the combinational group (*iv*) while all other treatments (*i.e.,* groups *i-iii*) reached only 0-20 % (Figure 1). With the high intensity SS stunning, 25 % of tilapia recovered VERs within 54-77 s, and 80 % of catfish within 43-269 s. Duration of VERs differed significantly between groups for both species (tilapia: $\chi^2(3)=8.657$, P=0.034; catfish: $\chi^2(3)=31.119$, P<0.001). In tilapia, the combinational group (*iv*) differed significantly from both HT (*i;* P=0.007) and SS low intensity (*ii;* P=0.042). In catfish, the combinational group (*iv*) differed from all other treatments (*i-iii;* P<0.001), and SS high intensity (*iii*) had shorter VER duration than SS low intensity (*ii;* $\chi^2(3)=5.145$, P=0.023).

**Conclusion**

In contrast to the results of our recent laboratory studies, the combination of electrical exposure, exsanguination and ice slurry immersion failed to prevent recovery in tilapia. While the same procedure achieved 100% insensibility in catfish, the rapid return of VERs, in some cases as early as within 43 s post-stunning, show that both throat cutting and immersion in ice slurry must occur immediately after stunning to prevent recovery during exsanguination and chilling. Until a clearly defined and practically feasible stun-to-stick interval can be established, the in-water electrical stunning as tested here cannot be recommended for either Nile tilapia or African sharptooth catfish. These findings highlight the need to refine stunning protocols under commercial conditions using species-specific parameters and precise timing to ensure humane slaughter of warm-water fish.

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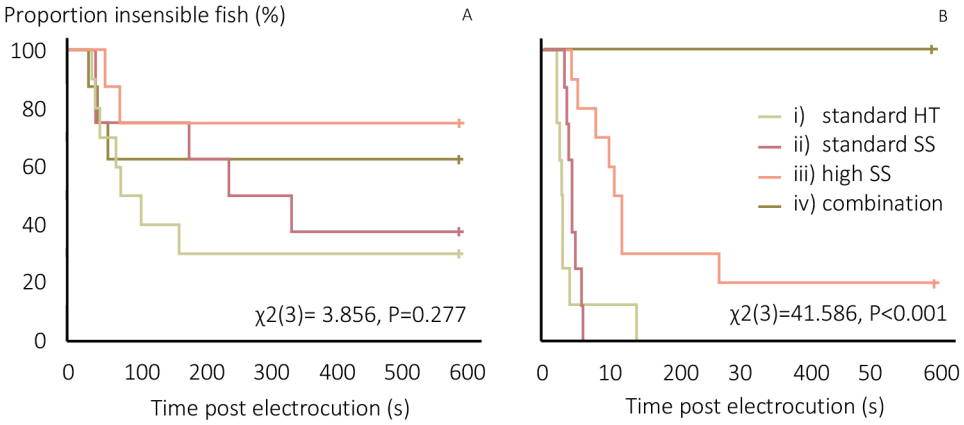


Figure 1. Kaplan meier curves showing the proportion of insensible fish post-stunning using 4 different protocols in A) Nile tilapia (*Oreochromis niloticus;* n=34) and B) African sharptooth catfish (*Clarias gariepinus;* n=36). The protocols (i-iv) refer to electrode configuration (head to tail=HT, and side to side=SS), and electrical intensity (standard or high). In the combination, electrocution was followed by exsanguination and ice slurry immersion. Cases were censored if no recovery occurred 10 min post-stunning (*i.e.,* at time 600 s), as indicated by the vertical line at time 600 s.

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# THE NORDIC MASTER'S PROGRAM IN SUSTAINABLE PRODUCTION AND UTILISATION OF MARINE BIORESOURCES: A MODEL OF SUCCESSFUL INTER- AND TRANSDISCIPLINARY COLLABORATION IN HIGHER EDUCATION

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In 2019, the Nordic master's program in Sustainable Production and Utilization of Marine Bioresources (MAR-BIO) was established by 4 Nordic universities: Gothenburg (Sweden), Nord (Bodø, Norway), Akureyri (left in 2022) and Hólar (both Iceland) in collaboration with the University of New England (Maine, USA). This unique trans- and interdisciplinary program aims to educate the next generation professionals in sustainable blue bioeconomy by combining natural sciences with environmental and social sciences, as well as economics and law. It introduces students to key aspects of the blue bioeconomy and its main industries in each partners' country. In Norway, focus is on salmon farming, in Iceland on the fishing industry and its sustainable use of natural resources and in Sweden on low-trophic aquaculture. All 3 countries jointly teach technical development of sustainable and innovative aquaculture systems, novel alternative feed ingredients and fish health and welfare in circular systems.

The program places strong emphasis on mobility and requires at least 30 ECTS to be earned outside the home university. The first course (15 ECTS) is a mandatory and hybrid course consisting of 3 modules of 1-2 weeks online, each followed by 1 week of on-site visits to each country. These visits include study tours to industrial partners and presentations of ongoing research (Fig.1). In the second semester, students apply for classes at 1 or more of the partner universities (15 ECTS) and the program is finalized by a 60 ECTS master's thesis course at any of the partner universities.

Now entering its 6$^{th}$ year, the master's program has grown from 5 students in the first cohort to 25. In this presentation, we will provide a detailed overview of the program, highlighting the transdisciplinarity and the future within this most rapidly growing and diversifying area of sustainable food production. We will show how the program offers a diverse network of contacts to the students both within the established industry, among innovative entrepreneurs and with authorities in the 3 partner countries. You will see the unique curriculum of the first mandatory course that lay the foundation for this successful cross-country collaboration in higher education, with potential to expand beyond the Nordic countries.

# NUTRITIONAL REPROGRAMMING OF HEPATIC IMMUNOMETABOLISM IN JUVENILE SPOTTED WOLFFISH (*Anarhichas minor*)

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## Introduction

Nutritional inputs can reprogram immunometabolic pathways, reshaping the balance between energy metabolism and immune function in cultured fish species. Modern aquafeeds differ markedly from natural diets, with protein and lipid sources increasingly derived from alternative ingredients. In parallel, functional feeds are being developed to enhance disease resistance and recovery, positioning nutrition as a central tool for health management. Advances in omics technologies now enable detailed characterization of diet-induced changes in immune and metabolic pathways, yet such knowledge remains limited in emerging cold-water species such as the spotted wolffish (*Anarhichas minor*). Here, we provide the first integrative evidence that protein-optimized diets reprogram hepatic immunometabolism in juvenile wolffish, revealed through combined histological, biochemical, metabolomic, and transcriptomic analyses.

## Materials and Methods

A seven-month feeding trial was conducted with juvenile wolffish (251.3 ± 22.1 g; 27.4 ± 1.7 cm) obtained from in-house breeding at Akvaplan-Niva (Tromsø, Norway). All procedures complied with Norwegian animal welfare regulations and were approved by the internal committee and the Norwegian Food Safety Authority (FOTS ID: 30374). Fish were assigned to three dietary groups: a control feed and two proprietary protein-optimized diets (Feed 1 and Feed 2, Skretting, Tromsø, Norway), each with two replicate tanks. Five fish per treatment were sampled at three time points, in addition to five baseline livers collected pre-trial. At each sampling, fish were euthanized with MS-222, and ~0.2 g of liver tissue was dissected, cleaned, weighed, and snap frozen. Semi-polar metabolites were extracted in methanol–water and analyzed by UPLC (Vanquish, Thermo Fisher Scientific) coupled to a high-resolution Orbitrap Exploris 240 MS under polarity switching. Stable isotope standards and pooled quality-control (QC) samples were included, with QC injections every sixth run. Data were processed with Compound Discoverer 3.3 and Skyline 23.1, and compounds annotated at three confidence levels (1–3) using accurate mass, isotope patterns, and MS/MS spectra. Statistical analyses combined univariate testing (Box-Cox transformation, t-tests, Benjamini–Hochberg FDR 0.05) with multivariate analysis (PCA) to identify diet-dependent clustering. Shotgun RNA sequencing, biochemical, and histological assays were also performed, with analyses still ongoing.

## Results and Discussion

Control fish achieved the highest growth (final weight 959.4 ± 84.2 g; length 53.0 ± 7.9 cm), while those fed protein-optimized diets displayed slightly lower final weights (832.8–869.0 g) and lengths (47.9–52.0 cm). Semi-polar metabolite profiling detected 1,739 compounds. Of these, 98 were confidently identified (Level 1), 78 annotated by accurate mass and retention time (Level 2a), 89 by MS/MS fragmentation (Level 2b), while 632 were assigned at lower confidence (Level 3) and 842 remained unclassified. PCA revealed clear separation of dietary groups, with Feed 2 producing the most distinct hepatic metabolic profile. QC samples clustered tightly, confirming high analytical reproducibility and that biological variance exceeded technical noise. Univariate analyses identified significant shifts in key metabolic pathways. Central energy metabolism was affected, with variation in pyruvic acid, lactic acid, glyceric acid, and glyoxylic acid pointing to altered glycolysis and TCA cycle activity. Several amino acids (alanine, glycine, threonine, valine) and derivatives (e.g., β-hydroxyisovaleric acid) were diet-responsive,

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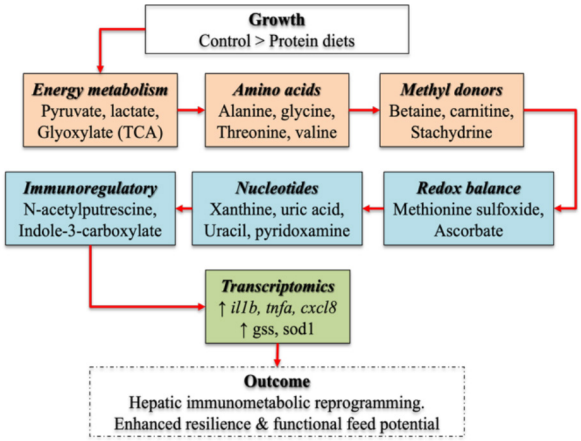


**Fig. 1** Diet-Induced Hepatic Immunometabolic Reprogramming in Spotted Wolffish

reflecting adjustments in protein turnover and nitrogen metabolism. Methyl donors and osmolytes such as betaine, carnitine, and stachydrine were strongly modulated, consistent with remodeling of one-carbon metabolism and lipid oxidation. Markers of redox balance, including methionine sulfoxide, methionine sulfone, and ascorbic acid, indicated enhanced oxidative stress buffering in fish fed experimental diets. Nucleotide derivatives such as xanthine, uric acid, uracil, and pyridoxamine were also affected, suggesting changes in nucleotide turnover and antioxidant defense. Furthermore, variation in polyamines (N-acetylputrescine) and tryptophan metabolites (indole-3-carboxylate) highlighted a potential dietary influence on immunoregulatory signaling. Preliminary transcriptomic screening supported these findings, with upregulation of pro-inflammatory cytokines (*il1b, tnfa, cxcl8*) and antioxidant regulators (*gss, sod1*), suggesting activation of cytokine signaling and redox defense pathways. Overall, protein-optimized diets modestly reduced growth but induced distinct hepatic immunometabolic reprogramming (**Fig. 1**). These metabolic signatures suggest prioritization of immune competence and physiological resilience over maximal biomass gain, supporting the potential of functional feeds for sustainable wolffish aquaculture.

## Conclusion

Protein-optimized diets reprogrammed hepatic immunometabolism in juvenile spotted wolffish, altering amino acid utilization, methyl donor pathways, redox balance, and immunoregulatory metabolites. While growth performance was slightly reduced, these nutritional interventions elicited distinct metabolic signatures associated with enhanced resilience and immune competence. To our knowledge, this represents the first integrative metabolic and transcriptomic evidence of diet-driven immunometabolic reprograming in spotted wolffish, providing a foundation for the development of functional feeds to support sustainable cold-water aquaculture.

## Acknowledgements

This research was supported by the Regionale Forskningsfond (RFF) Arktis (grant 341234) through the project *"A collaborative platform to support upscaling of spotted wolffish aquaculture."* We thank the staff of Akvaplan-Niva (Tromsø, Norway) for fish rearing and husbandry, and the technical staff of Nord University for their valuable assistance.

# LONG-CHAIN POLYUNSATURATED FATTY ACID BIOSYNTHESIS IN THE SALMONIDS *Salmo trutta* AND *Thymallus thymallus*

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**Introduction**

Long-chain polyunsaturated fatty acids (LC-PUFAs) play a crucial role in brain and eye development and function, form essential components of cell membranes, and possess anti-inflammatory properties. The main biologically active LC-PUFAs are arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). In the absence of enzymes required for the synthesis of LC-PUFAs from oleic acid (18:1n-9), vertebrates must obtain PUFAs from their diet (1). However, some vertebrates can convert C18 PUFAs, such as alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), into biologically active LC-PUFAs.

The capacity for this conversion is species-specific and depends on the presence and expression of genes encoding fatty acid desaturases (Fads) and elongases (Elovl) (2). In fish, the ability to meet physiological demands for LC-PUFAs through elongation and desaturation of C18 PUFA precursors varies among species. This variability is influenced by both genetic factors and environmental conditions, including diet.

In aquatic food webs, the distribution of LC-PUFAs—particularly n-3 LC-PUFAs—follows a clear gradient from nutrient-poor headwater streams to lakes rich in n-3 LC-PUFAs. However, in freshwater ecosystems, the availability of prey rich in n-3 LC-PUFAs is rapidly declining due to anthropogenic impacts such as climate change and land-use alteration. Consequently, the ability to biosynthesise n-3 LC-PUFAs is of critical importance for the long-term survival of species.

**Material and methods**

Our model species are brown trout (Salmo trutta), represented by two ecotypes (lake and stream trout), and grayling (Thymallus thymallus), which inhabit distinct habitats. To evaluate the potential for LC-PUFA biosynthesis, we examined the genomic presence and tissue-specific expression of candidate genes in hatchery-reared individuals maintained under identical conditions to minimise environmental effects. The analysed tissues included brain, pyloric caeca, liver, heart, and muscle.

Candidate genes (FADS2, FADS6, Elovl2, Elovl4a, Elovl4b, Elovl5, Elovl8a, Elovl8b, ehhadh, Hacd1) were selected based on previous literature. Their presence and copy number were first determined using Ensembl (Release 111) for brown trout and BLAST searches against the available grayling genome. For most candidate genes, multiple copies were identified in both genomes. Where possible, gene expression analyses were conducted separately for each copy.

**Results and conclusions**

The genomes of S. trutta and T. thymallus contain multiple copies of genes that encode enzymes involved in fatty acid synthesis. The expression profiles of these gene copies are tissue-specific in both species.

Some candidate genes showed a similar pattern of expression across different copies, while others showed clear overexpression of one copy. Differences in the expression profiles of certain genes/gene copies were observed between the two S. trutta ecotypes, which may indicate adaptation to different environments (stream versus lake) and the diets of wild populations.

Multiple amino acid (AA) substitutions were found between gene copies in the aligned protein sequences. For example, three copies of the FADS2 gene are present in S. trutta, with pairwise distances between them ranging from 6.6% to 8.5%. AA substitutions were also found in conserved regions, such as haem-binding motifs, the cytochrome b5-like domain and transmembrane motifs.

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# ESTIMATING THE WATER FOOTPRINT OF FISH FEED IN CLOSED-LOOP AQUAPONIC SYSTEMS (CLA SYSTEM)

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## Introduction

Closed-loop aquaponic water recirculation systems (CLA) are innovative, integrated food production systems that combine recirculating aquaculture with hydroponic plant cultivation, using the same water in a continuous loop. In these systems, nutrient-rich wastewater from fish tanks is biologically filtered and recirculated to hydroponic units, where plants absorb dissolved nutrients, thereby purifying the water before it returns to the fish tanks (Maucieri et al., 2017). This symbiotic relationship enables efficient nutrient recycling, minimizes water consumption—often reducing usage by 90–99% compared to traditional aquaculture—and significantly lowers environmental discharge and pollution (Ahmed et al., 2024; Badiola et al., 2012). CLA systems maintain optimal water quality for both fish and plants through advanced filtration, oxygenation, and real-time monitoring technologies, supporting high-density fish production and robust plant growth (Lal et al., 2024). The closed-loop design not only enhances resource efficiency and sustainability but also offers economic and environmental benefits, such as lower chemical use, and the potential for local food production in urban or resource-limited settings (Lal et al., 2024). Fish feed water footprint is a critical component in the sustainability of aquaponics. Production-weighted average feed water footprints for commercial aquafeed, for the major farmed species, were estimated at 1629 $m^3/t$ (green), 179 $m^3/t$ (blue), and 166 $m^3/t$ (grey), with common carp among the top contributors to global aquafeed water use (Pahlow et al., 2015). The current study aims to assess the blue, green, and grey water footprint of fish feed (indirect water consumption) in a closed-loop aquaponic water recirculation system (CLA) containing common carp (Cyprinus carpio) and lettuce (Lactuca sativa), following the Water Footprint Network methodology.

## Material and Methods

Common carp (*C. carpio*) grown in a laboratory CLA system with lettuce (*L. sativa*) was fed four meals per day at six-hour intervals. The 45-day trial was carried out at the laboratory of Aquaculture and Aquatic Animal Diseases, School of Veterinary Medicine at Aristotle University of Thessaloniki. Three different diets were used: the control diet (C), which contained 15% fishmeal, the *Hermetia illucens* diet (B), which substituted 50% of the protein in fishmeal with *Hermetia* meal, and the plant residues diet (F), which substituted 50% of the protein in fishmeal with a 2:1:1 mixture of *Hermetia* meal, *Hermetia* frass, and hydroponic by-products. By calculating the water footprints of the feed ingredients used to create the three distinct fish diets, the blue, green, and grey water footprints of fish feed (indirect water consumption) of the different diets were assessed. More precisely, fishmeal, Hermetia meal, Hermetia frass, dried hydroponic by-products, poultry meal, rapeseed meal, soybean meal, sunflower meal, corn gluten meal, wheat meal, and rapeseed oil were the primary feedstuffs utilized for the diet compositions.

## Results and Discussion

The Water Footprint Assessment Manual (Hoekstra et al., 2011) following the Water Footprint Network approach, enables spatially explicit quantification of water consumption and pollution associated with crop-derived feed ingredients, considering the three components of the water footprint (blue, green and grey) (Mekonnen and Hoekstra, 2011). Integrating these estimates into closed-loop aquaponic systems highlights the importance of feed sourcing and nutrient management for minimizing indirect water consumption and environmental impact, supporting the development of more sustainable aquaponic production of carp and lettuce.

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**Acknowledgements**:

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# TEMPORAL DYNAMICS OF TANK BIOFILM ACROSS DIFFERENT DIETARY TREATMENTS AND AQUACULTURE SYSTEMS

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## Introduction

While biofilm formation in biofilters has been extensively studied, microbiota attached to other surfaces within recirculating aquaculture system (RAS), such as tank walls, remain largely unexplored. This is particularly relevant, as sloughed biofilm can contribute to microbial dispersal throughout the system via water recirculation. Gaining insights into the factors influencing biofilm development is therefore crucial for managing system microbial dynamics and potential pathogen reservoirs. To this end, this study investigated the effects of system type and diet on the temporal dynamics of tank biofilm over an eight-week period.

## Materials & Methods

Three mariculture systems with different water refreshment rates were examined: a flow-through system (FTS), a high refreshment (HR-RAS), and a low refreshment RAS (LR-RAS). Each system consisted of four tanks evenly stocked with juvenile gilthead seabream (*Sparus aurata*). To assess the effect of diet, fish in each tank were fed restrictively one of two aquafeeds in duplicate: a diet rich in non-starch polysaccharides (NSP-DIET) or a starch-rich diet (S-DIET). Water quality and biofilm development were analyzed at the tank level at Week 0, 2, 5, and 8. Water samples were filtered at 0.22 μm immediately after collection, and biofilm samples were obtained by swabbing tank walls. Water physicochemical parameters were assessed using standard analytical methods. Microbial abundance was measured via quantitative polymerase chain reaction (qPCR). Microbial community composition was determined through full-length 16S rRNA gene sequencing using the ONT MinION device; sequences were taxonomically assigned using the SILVA v148.1 database and further analyzed with the R microeco package.

## Results & Discussion

By the end of the trial, tank biofilm in the LR-RAS showed the highest microbial abundance and a trend toward higher organic carbon levels, indicating that nutrient accumulation promotes biofilm growth. Alpha diversity also varied most in the LR-RAS, with a short-term decline linked to the NSP-DIET. This contrasted with trends observed in the tank water—where alpha diversity fluctuated more under higher water refreshment rates—suggesting that biofilm and water microbial dynamics are likely independent of each other. Beta diversity in tank biofilm shifted over time across all systems, with each maintaining distinct prokaryotic communities throughout. Despite these shifts, the relative abundance of dominant taxa remained stable and included no known gilthead seabream pathogens (Fig1A), even though potentially pathogenic bacteria (e.g. *Vibrio*, *Coxiella*) were detected in water from the two RAS. Differences among systems were partly explained by physicochemical water quality parameters, with organic carbon and nitrogen levels strongly shaping biofilm prokaryotic communities in the two RAS. In the LR-RAS, diet also influenced biofilm community structures (Fig1B). Nonetheless, it did not promote the presence of common sulphate-reducing (i.e. Desulphobacteroda) or off-flavor producing (e.g. *Oscillatoria perornata*, *Anabaena spp.*, and *Pseudanabaena*) bacteria, which remained at low relative abundances in all systems, despite their frequent presence in previous biofilm studies (Houle et al., 2011; Rojas-Tirado et al., 2021). Overall, the present findings highlight that environmental conditions are the primary drivers of biofilm development in aquaculture systems, while diet plays a secondary but notable role under low water exchange.

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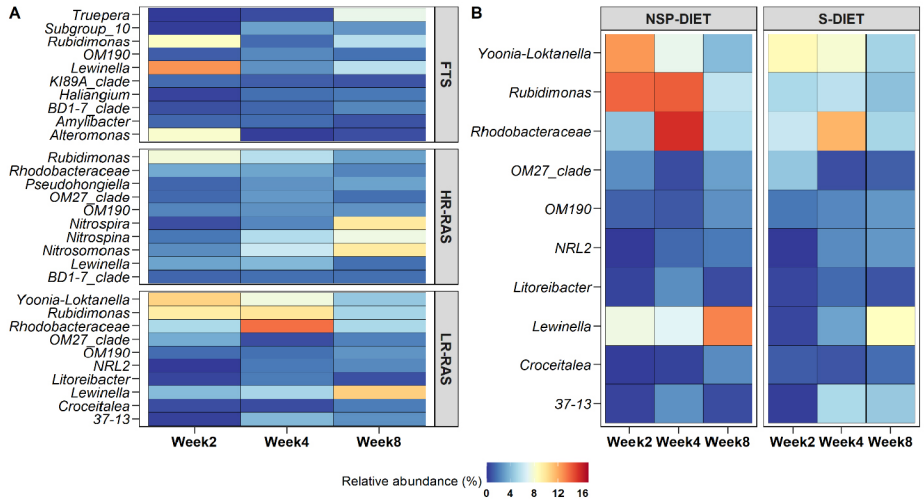


Fig1. Temporal mean relative abundances of the 10 most prevalent genera in the tank biofilm A) as observed across systems, with samples from both diets being pooled together, and B) as found in the LR-RAS, with samples being grouped per diet.

**Funding**

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# CIRCULAR PRODUCTION OF PACIFIC WHITELEG SHRIMP (*Litopenaeus vannamei*) UNDER HIGH STOCKING DENSITIES

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## Introduction

As global water stress intensifies, optimizing water management becomes critical. In this context, the CisWEFE-NEX project seeks to integrate innovative solutions that optimize the interconnections between water, energy, food, and ecosystems, promoting the sustainable use of natural resources. In this framework, a low-footprint recirculating aquaculture system (RAS) will be engineered to produce whiteleg shrimp at high stocking densities. The system will operate with minimal water exchange, with inputs and outputs linked to onsite operations to ensure resource circularity.

## Materials & Methods

The ~40 $m^3$ RAS (Fig.1A) will be installed at the CisWEFE-NEX demonstration site in Algarve, Portugal, and will consist of three culture tanks (10$m^3$). Tanks will feature a multi-platform setup connected to an air-lift pump (Fig.1B), providing increased surface area for shrimp colonization and efficient in-tank water circulation. At a system level, high water recirculation will be achieved via a state-of-the-art water treatment unit, including a drum filter (60 μm), a moving-bed bioreactor (1.5 $m^3$ RK plastic bio media; 750$m^2/m^3$), a custom packed-column degasser (0.14 $m^2$), a sump (0.7 $m^3$), and a low-head oxygen cone (max 50 $m^3$/h). Discharge water will be directed to an onsite prototype farm for seaweed cultivation, while collected sludge will be supplied to an onsite biogas plant for heat and energy production, which will in turn power the RAS. Under this setup, shrimp will be grown from post-larvae (~1gr) to market size (~25 gr) in three annual batches using a commercial feed. Throughout the project, production metrics, including survival and growth rates, will be monitored to assess the system's capacity. Additionally, both CAPEX and OPEX will be analyzed to evaluate the economic viability of the system.

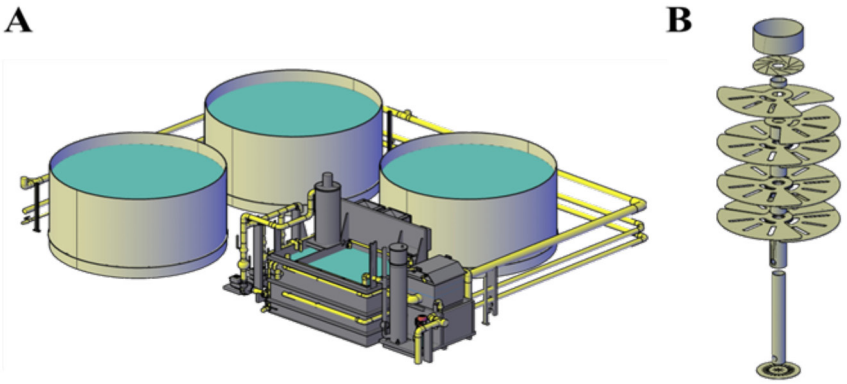


Fig1. A) 3D schematic of the RAS outlining its key components. B) Top-down layout of the in-tank platform arrangement, showing the centrally positioned air-lift pump.

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**Expected outcome**

This technologically adapted system for shrimp farming aims to achieve stocking densities of up to 20 kg/m$^3$, which is twice the level typically applied in conventional shrimp systems to suppress cannibalism (Romano & Zeng, 2016). Growth and survival are expected to be on par with those observed in intensive operations (Thakur et al., 2018), as high-quality feeds will be used and optimal water quality standards will be maintained. Due to the continuous solids removal, the system's oxygen demand is projected to be lower compared to common biofloc systems with high microbial, heterotrophic activity. The high production capacity per unit volume of water is expected to reduce capital costs per unit of shrimp produced, offering a cost-efficient solution to shrimp farming in RAS. Operational costs will also be minimized through the use of energy-efficient equipment and local, circular energy resources. Overall, the proposed system seeks to enhance both the efficiency and profitability of RAS shrimp farming while aligning with emerging sustainability and circular economy principles.

**Funding**
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# SUPPLEMENTED FEEDING AS A METHOD TO MODULATE IMMUNE RESPONSES AND IMPROVE THE RESPONSE TO VACCINATION IN AQUACULTURED FISH

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For many years now, diet has been identified as a pivotal conditioner of health and immune status in both mammals and fish. Yet the extent to which diet composition or diet supplementation with immunostimulants affects the response to vaccination has been scarcely studied. In this study, we have carried out a series of different feeding experiments in rainbow trout (Oncorhynchus mykiss) to determine how diet composition can affect the antibody response to vaccination. For this, fish were fed the different supplemented diets or a control diet for 30 days. At that point, fish were immunized with different antigenic models through different administration routes and at day 30 post-immunization, fish were sacrificed and systemic specific antibody responses analyzed by ELISA. Antibody affinity was also determined in some cases. These experiments included diets supplemented with methionine or enriched with β-glucans or the probiotic microorganism Bacillus subtilis. The diets tested were selected based on preliminary in vitro experiments that demonstrated the capacity of these substances to increase B cell function in rainbow trout. Our results demonstrate that an optimized diet can improve the performance of vaccines in fish, being this especially useful for underperforming mucosal vaccines. The results presented will be useful for the design of holistic vaccination protocols that will not only focus on antigen optimization or administration regimes, but that will also include diet composition as an important factor to influence the outcome of the immunization strategy.

# EFFECTS OF PRODUCTION TEMPERATURE, WATER CURRENT VELOCITY, AND THEIR INTERACTION ON FARMED ATLANTIC SALMON (*Salmo salar*) STRESS RESPONSES AND WELFARE

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## Introduction

Farmed Atlantic salmon (*S. salar*) mortalities have increased in recent years, occurring during both the freshwater (FW) and seawater (SW) production phases. Meanwhile, suboptimal rearing and transfer strategies between FW and/to SW can greatly influence fish resilience. For instance, seasonal mismatches in the thermal environment during transfer can have pronounce effects on fish welfare and challenge abilities to adjust to the novel SW environment. At the same time, high water velocities are increasingly favoured in aquaculture settings due to reported benefits on fish health, including improved stress coping and cardiovascular fitness. However, the interactions between such training procedures and temperature and their effects on stress functions critical for farmed salmon welfare is still poorly known. Therefore, this study aimed to evaluate how temperature, water current velocity and their interaction influences farmed salmon welfare by monitoring stress responses and key neural markers of brain plasticity, with special reference to the sensitive FW-SW production phase.

## Materials and methods

Atlantic salmon parr (initial body weight ~ 40g) were allocated in duplicates between two water velocities (body length [BL] / second [s]): **1)** H (1.2 BL/s) and **2)** L (0.6 BL/s). The fish were maintained under these conditions at 10°C in FW RAS for 8-weeks. During this period fish were smoltified according to standard industry practice, followed by SW transfer of smolts in the same respective water velocities. To investigate the effect of abrupt temperature changes that often results from transfer from land-based sites to sea-cages, we investigated three distinct temperature regimes (**1)** 8°C [8-H & 8-L], **2)** 10°C [10-H & 10-L] and **3)** 14°C [14-H & 14-L]). The fish remained under these conditions for an additional 8-weeks before final sampling. At the end of both FW and SW phases, 20 fish from each treatment (N=5 per tank) were sampled for blood and brain tissue. In each treatment, 10 fish were immediately euthanized (pre-stress) and the other 10 were subjected to an acute challenge test (ACT), by high density, to monitor stress response capacities (ACT; 15 min. at 300 kg. $m^{-3}$, followed by 45 min. recovery). Plasma levels of cortisol were measured using ELISA, and ions (e.g., sodium, chloride) and energy metabolites (e.g., glucose, lactic acid) analysed using Horriba Pentra 400. These were compared to telencephalic expression changes in genes involved in the stress response (mineralocorticoid receptor; *mr*, glucocorticoid receptors; *gr1* and *gr2*) and neural plasticity (e.g., neurogenic differentiation factor; *neurod*, e.g., immediate early gene [IEG]; *c-fos*). Data was subjected to one- and two-way ANOVAs ($p < 0.05$).

## Results & Discussion

**Effect on growth performance:** Fish final average body weight was ~ 415 g. In FW, the specific growth rate (SGR) was unaffected by water velocity. In SW, SGR increased towards higher temperature regimes (8°C:~1.61, 10°C: ~1.68, 14°C:~1.86). Only at 14°C was the SGR further improved using high-water velocity (1.2 b/s).

**Plasma ions and metabolites:** In FW, plasma glucose, lactic acid, and $Na^+$ levels were lower in response to the high-water velocity (Fig. 1). This may indicate larger energetic demands and disturbed ion-homeostasis, effects consistent with osmo-respiratory compromise. In SW, the pre-stress 10-H group at 10°C showed a general elevation in plasma ions suggesting increased osmoregulatory challenges (Fig.1). Conversely, lactic acid levels were highest in the 14°C group following the ACT, while the high-water velocity seemed to elevate lactic acid build-up at pre-stress status in the 14-H group (Fig. 1). This build-up in lactic acid may be a consequence of thermal increases in metabolic rate alongside higher energetic demands to sustain swimming activities that elevate anaerobic forms of metabolism.

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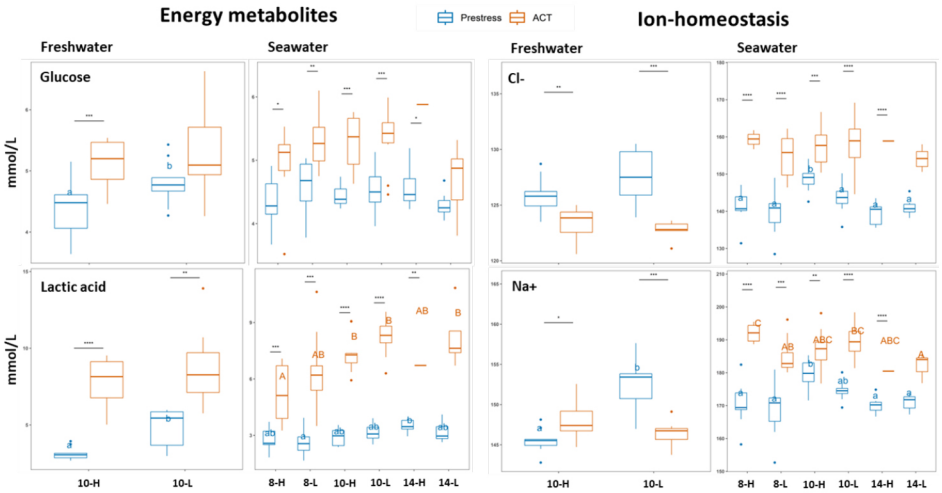


**Fig. 1.** Plasma stress responses at the end of the FW and SW phases. Lower- and upper-case letters denote significant differences among treatments, while asterisks indicate significant differences between the two stress states within each diet (*, **, ***, **** denote $p < 0.05$, 0.01, 0.001, and 0.0001, respectively).

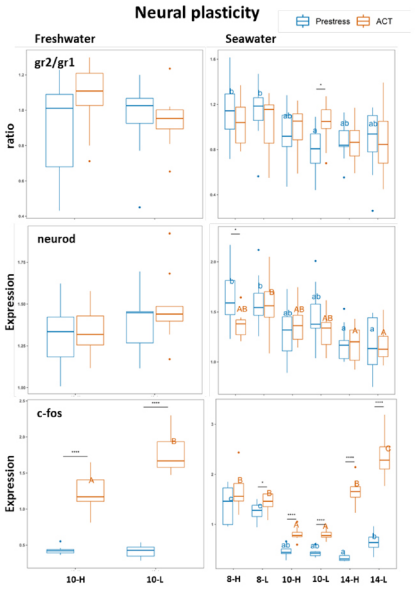


**Fig. 2.** Neural responses in the telencephalon at the end of the FW and SW phases. The rest of the legend is as in Fig. 1.

**Neural functions:** A gradual suppression in corticosteroid receptors (*mr*, *gr2*) and neural plasticity (*neurod*) was observed with increasing temperature, consistent with our prior findings (Fig. 2) (Tang et al., 2022). However, the effect of water current velocity was less apparent. In FW, the response of *c-fos* to the ACT appeared lower in the fish that had experienced the highest velocity strength, which could suggest reduced stress coping, while, in SW, this difference was only observed at the highest temperature that agrees with the more stressful effects seen in plasma lactic acid levels in the 14-H group.

**Conclusion:** The effect of water velocity in SW was less apparent compared to that of temperature. Since growth was maintained well throughout all treatments, this suggests that farmed salmon still handled the tested temperature-water velocity treatments well without marked effects on welfare status.

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# EVALUATING THE DIETARY INCLUSION OF A THYMOL-BASED BLEND TO MODULATE RESISTANCE AND SURVIVAL AGAINST *Yersinia ruckeri* IN RAINBOW TROUT

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*Yersinia ruckeri*, the causative agent of enteric redmouth disease (ERM), is a common pathogen that causes substantial economic losses in salmonid aquaculture. This study evaluated the efficacy a thymol-based blend – Prototype TCS – in reducing mortality on juvenile rainbow trout in following immersion challenge with *Yersinia ruckeri*. Two practical diets were manufactured to contain 2000 ppm of Prototype TCS and no additive (Control) and fed to 320 trouts divided in 16 tanks ($n$=8). After four weeks of feeding, the fish were challenged with *Y. ruckeri*. Fish fed diets containing Prototype TCS effectively reduced post-challenge mortality by 15.6% and hazard ratio by 29.3% (P<0.05 -Figure 1). Additionally, fish fed Prototype TCS had a significantly lower prevalence (P<0.05) of pathological signs in survivors, supporting enhanced disease robustness. Our results demonstrated that including Prototype TCS in rainbow trout diets significantly increased resistance against ERM. This additive could therefore serve as promising alternatives to reduce antibiotic reliance and support more sustainable aquaculture practices.

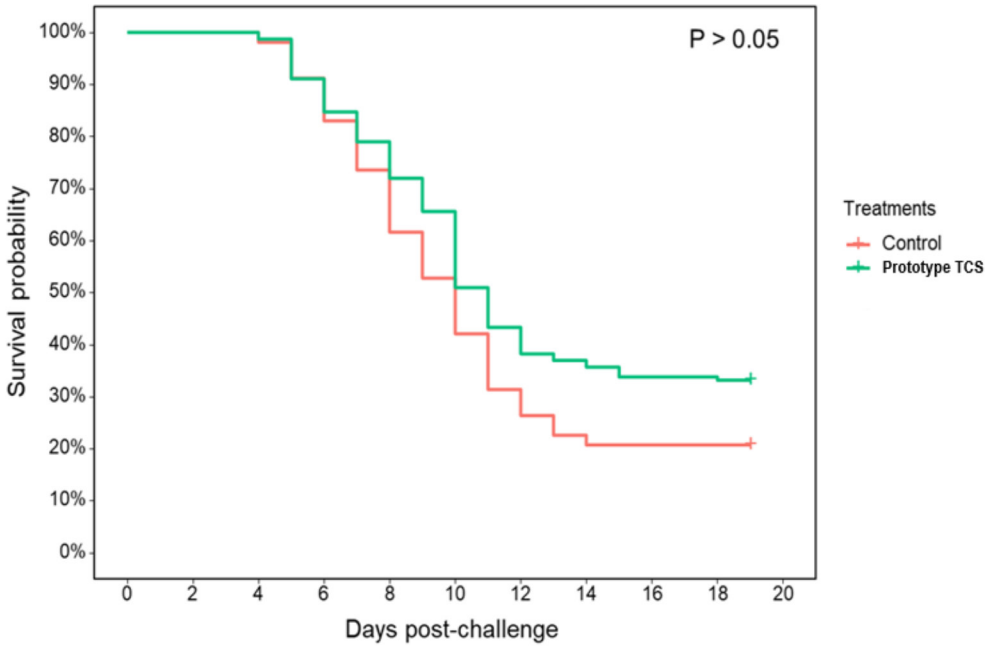


Figure 1. Survival curves of rainbow trout challenged with *Y. ruckeri* fed diets containing botanical-based blend.

# UNLOCKING PROTEIN DIGESTIBILITY IN EUROPEAN SEABASS (*Dicentrarchus labrax)* WITH PLANT EXTRACTS TO REDUCE FEED COSTS

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Fishmeal (FM) is the primary protein source in aquaculture feeds, but its price has risen due to overfishing and climate change reducing supply. Alternatives like plant-based proteins, insect meals, and single-cell proteins are being explored, but their lower digestibility can hinder growth and feed efficiency. Enhancing their digestibility is key to meeting the nutritional needs of farmed species.

Economix is a plant-based feed additive produced by TECHNA (Couëron, France) that aims at enhancing protein digestibility. It offers a promising solution for reducing FM use and substituting it with more affordable plant proteins.

A digestibility trial was held at Sparos, Portugal, to evaluate the effect of Economix on apparent digestibility coefficients (ADC) of nutrients in European seabass (*Dicentrarchus labrax*). The trial comprised two experimental diets, both containing 0.02% yttrium oxide as an inert digestibility marker.
Control diet: 30% FM
Economix diet: 28% FM + 0.2% Economix

The Economix diet reduces FM by 2% compared to the Control diet, replacing it with plant proteins (soybean meal, wheat) and 0.2% Economix additive. This reformulation achieves a cost reduction of -1.78% compared to the Control diet.

Each diet was tested in triplicate groups of 13 fish (mean body weight: 73 ± 5 g), maintained in 60 L tanks at a constant water temperature of 20.0 ± 0.43°C. Feces were collected in tanks equipped with a fecal decantation column (Guelph system).

The results showed that the inclusion of 0.2% Economix significantly improved the amino acids digestibility of 13 out of the 17 analyzed amino acids, including lysine, methionine, and threonine ($p < 0.05$). This trial also indicated numerically higher values, although not statistically significant, for protein, fat and energy digestibility.

To conclude, Economix, with its ability to enhance amino acid digestibility, offers a viable solution by enabling the reduction of fishmeal and the incorporation of cheaper, more sustainable protein sources, while maintaining optimal performance in fish and shrimp.

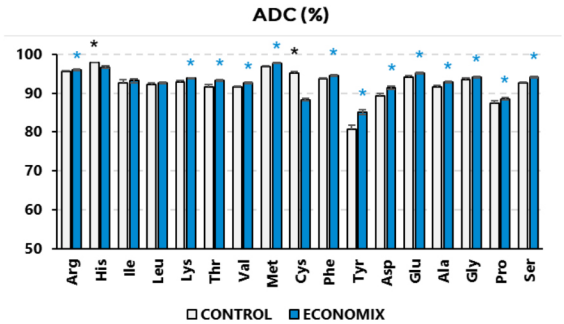


Figure 1. Effect of Economix on amino acid digestibility of European seabass *Dicentrarchus labrax* at Sparos, Portugal

# LONG-TERM COMPARISON OF GREEN AND RED MACROALGAE EXTRACTS AND β-GLUCANS ON THE DISEASE RESISTANCE, GILLS INTEGRITY AND GROWTH PERFORMANCE OF RAINBOW TROUT

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This study aimed to evaluate the efficacy of green and red macroalgae extracts and β-glucans on the disease resistance, gills integrity, and growth performance of rainbow trout (*Oncorhynchus mykiss*) in long-term use. An algae-based marine sulphated polysaccharides (MSP) extracts, from Ulva sp. and Solieria chordalis, and 60% purified yeast-based β-glucans available in the market were used in the feeding trial. Fish weighting 27.6 g were reared at 14-17 °C, and average dissolved oxygen above 6 mg/L. Fish were divided into four different dietary treatments and 6 replicates (tanks of 70 L): Control, Algae MSP 0.3%; β-Glucan A 0.1% and β-Glucan B 0.1% produced as pelleted feed of 3mm. Fish were stocked at a density of 12 fish per tank (72 fish per treatment) and fed twice a day for 100 days. At the end of the feeding trial, 8 fish per treatment were sampled for blood-based innate immune response analysis. Another 6 fish per treatment were exposed to a bacterial solution containing *Aeromonas hydrophila* and *Pseudomonas anguilliseptica* via immersion for 24 hours, and gills were sampled after 72 hours for histopathological evaluation by a specialized laboratory using a diagnostic scoring system. Additionally, a third group of 30 fish per treatment was intramuscularly challenged with the same bacterial solution, and survival was monitored for 7 days. Data were analyzed using one-way ANOVA followed by SNK post hoc test ($p<0.05$), and survival was analyzed using the Kaplan-Meier method (SIGMA PLOT 11.0, Systat Software, San Jose, CA, USA). Trout fed the Algae MSP exhibited the best overall performance, with a 13% higher weight gain (45.8 g vs. 40.5 g; $p=0.017$), increased lysozyme activity (117 vs. 79 U/mL; $p=0.016$), and enhanced complement activity (40.5% vs. 2.4%; $p <0.001$) and were the only group which differed from control for survival 40% and 10%, respectively (+400%). Likewise, higher complement hemolytic activity was also observed in fish fed β-Glucan B (45.7%). Fish fed Algae MSP showed the lowest gills histopathological score among the challenged groups, indicating a 38% reduction in gill damage, 0.85, 0.53, 0.65, and 0.71, respectively. In conclusion, Algae MSP demonstrated the most consistent overall performance, excelling in growth, innate immunity, survival and gills integrity. The results highlighed the algae-based solution as a tool for strengthening natural defenses in aquaculture due to its broader benefits in long-term use.

# SOURCES OF HEPATIC GLYCOGEN TRACED BY $^{13}$C-NMR IN FRUGIVOROUS AMAZON TAMBAQUI (*Colossoma macropomum*)

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## Introduction

The use of plant-based feed ingredients proportionally increases the amounts of plant-derived carbohydrates, including digestible carbohydrates, starches and sugars in aquafeeds. Despite not being essential, the energy derived from carbohydrates can potentially have protein-sparing effect. However, carnivorous fish display several digestive, enzymatic or hormonal limitations in the metabolism of carbohydrate-rich feeds. Tambaqui is an Amazon fish species (*Colossoma macropomum*) with herbivorous/frugivorous feeding habits, very different from the marine carnivorous species commonly farmed (Ferrari et al., 2022). The study aims to assess how carbohydrates are assimilated at the hepatic glycogen of tambaqui through the injection of an intraperitoneal [U-$^{13}$C]glucose or [U-$^{13}$C]fructose load (2g kg-1). $^{13}$C-NMR analysis of hepatic glycogen was used to determine the contribution of the load to glycogen synthesis via direct and indirect pathways at 24h post-injection under fed and fasted conditions.

## Materials and methods

All animal procedures for handling and sampling were performed as previously described (Ferrari et al., 2022). Briefly, a total of 90 fish were randomly distributed into nine 900 L tanks with average body weight per tank 157.8 ± 5.2 g in a closed freshwater circulation system. The fish were acclimated for 10 days and fed to satiation 3x day with a commercial feed. Six tanks were assigned for fed fish (FED, 60 fish, 10 fish per tank): three for IP-injected with glucose (GLU, 30 fish, 10 fish per tank), and three for IP-injected with fructose (FRU, 30 fish, 10 per tank); these continued the feeding regime for another 10 days. Three tanks were assigned for fasted fish (FAST, 30 fish, 10 fish per tank); these were feed-deprived for 10 days prior to injection with [U-$^{13}$C]glucose or [U-$^{13}$C]fructose load (2g kg-1) Fish were processed 24h after injection, under anaesthesia, weighted, sampled for blood (Ferrari et al., 2022), and sacrificed by cervical section. The liver was excised, weighed, freeze-clamped in liquid nitrogen, and stored at -80ºC until further processing. Hepatic glycogen was extracted after MTBE extraction and derivatisation for NMR experiments was performed as previously described (Viegas et al., 2012).

## Results

Enrichment of the load with [U-$^{13}$C]glucose or [U-$^{13}$C]fructose will generate glycogen 13C-isotopomers that can be attributed to direct and indirect pathways activities (Viegas I et. al 2022). The 10-day fasted tambaqui, synthesized more hepatic glycogen from the [U-$^{13}$C]glucose load than fed tambaqui ($p<0.001$, 24.9±7.9% vs. 11.5±3.8%, respectively). The indirect pathway fluxes were similar in both fasted and fed conditions (ns, 6.1±1.7% vs. 6.4±2.9%, respectively). Considering the [U-$^{13}$C]fructose load, an indirect pathway substrate, can provide information regarding fluxes originating at triose-P level or arising from Krebs cycle. In both fluxes, the fasted tambaqui significantly increased the fluxes towards hepatic glycogenesis (triose-P level $p<0.001$, 55.1±7.3% vs. 31.0±8.2%; Krebs cycle level $p<0.05$, 12.8±2.1% vs. 10.0±2.6%). In both assays we were able to quantify the fraction of direct pathway glucose and fraction of indirect pathway fructose metabolized by pentose phosphate pathway.

**Discussion and Conclusion**

Our results clearly show that the direct pathway contributed more to glycogen synthesis than indirect pathway contributed less to glycogen synthesis from a glucose load in fasted tambaqui. The results show a clear contrast with carnivorous fish, which rely almost exclusively from indirect pathway (Viegas I et al. 2012). Furthermore, tambaqui is able to metabolize large amounts of fructose towards glycogenesis through indirect pathway. Such results offer great insight on the mechanisms employed by tambaqui to assimilate carbohydrates and will allow to optimize fructose-rich ingredients for aquaculture.

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# BIOFILM FORMATION OF *Tenacibaculum maritimum*, A FISH PATHOGENIC BACTERIA, TO EVALUATE THE ANTIMICROBIAL ACTIVITY OF FISH SKIN MUCUS

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## Introduction

*Tenacibaculum maritimum* is a Gram-negative filamentous bacterium that accounts for the majority of tenacibaculosis cases in marine fish species (Mabrok et al., 2023). Although biofilm formation is presumably a necessary step in the establishment of the infection by *Tenacibaculum* (Levipan et al., 2019), biofilms have not been usually included in studies assessing the antibacterial activity of fish skin mucus against this pathogen. In this context, the present study aimed to establish a simple and reproducible protocol for the formation and quantification of *T. maritimum* biofilms that will allow a more realistic approximation when studying the antibacterial properties of different compounds against this pathogen. For this purpose, a first attempt to elucidate the effects of gilthead seabream (*Sparus aurata*) skin mucus on *T. maritimum* planktonic and biofilm growth was included.

## Materials and methods

This study developed an evaluation of skin mucus antibacterial activity on planktonic cultures, on biofilm formation and on mature biofilms of *Tenacibaculum maritimum* (CECT 4276 strain from the Spanish Type Culture Collection University of Valencia, Spain). To do this, an experimental setup for the biofilm formation has been developed. A pool of homogenized skin mucus of gilthead seabream juveniles (*S. aurata*) was created and three different dilutions were tested in the antibacterial assays. The activity of skin mucus on biofilms was monitored through Crystal Violet staining (biomass) and resazurin assay (metabolic activity).

## Results

The results obtained demonstrated that seabream mucus significantly impairs the planktonic growth of *T. maritimum* in a concentration-dependent manner: non-diluted mucus (9.6 mg $ml^{-1}$) completely inhibited *T. maritimum* growth, 1:2-diluted mucus initially caused high inhibition that was reduced during the later hours of culture, and a 1:5 dilution of the mucus resulted in an almost complete loss of antibacterial activity (Figure 1).

The antimicrobial effect of the fish skin mucus on the biofilm formation showed similar results to those obtained with planktonic cells. Non-diluted mucus strongly inhibited biofilm formation: a high reduction in biofilm biomass was observed and no metabolic activity was detected. The inhibitory effect decreased when 1:2-diluted mucus was used and lost with the 1:5-dilution. Undiluted gilthead seabream skin mucus demonstrated significant effects on mature biofilms, promoting substantial biofilm biomass dispersion (40%) and reducing the metabolic activity by approximately 50%. In contrast, diluted mucus appears not to affect biofilm biomass and viability in the dispersion assay.

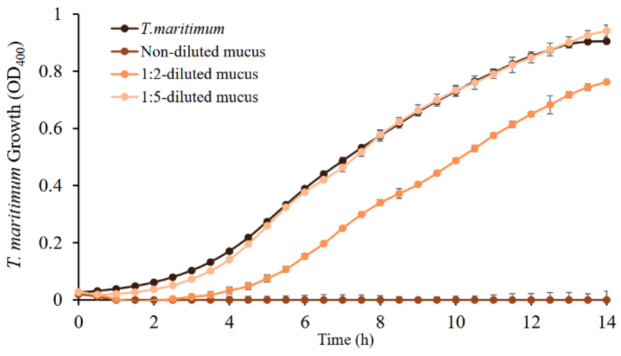


**Figure 1.** Skin mucus antibacterial activity against planktonic cultures of *T. maritimum*. Growth of *T. maritimum* incubated without or with different dilutions of gilthead seabream skin mucus.

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**Discussion and conclusions**

Bacterial biofilms constitute significant challenges in medical and ecological contexts due to their remarkable ability to resist antibiotic treatments, leading to persistent infections (Flemming et al., 2016). This study aimed to provide a platform for studying the potential antimicrobial effects of fish skin mucus on the formation and dispersion of biofilms of *T. maritimum*. The results showed that the mucus inhibited biofilm formation in a concentration-dependent manner. Considering the effect of skin mucus on planktonic growth, the inhibition of biofilm formation observed could be a consequence of the inhibition of total bacterial growth in the presence of the skin mucus. Our results demonstrated that, in addition to inhibit biofilm formation, seabream skin mucus could alter previously formed *T. maritimum* biofilms. However, as expected, the impact of skin mucus on the disaggregation of *T. maritimum* pre-formed biofilms was found to be less pronounced, evidencing the high resistance that these structures confer to the bacterial cells. Assessing how fish culture conditions influence the host's vulnerability to bacterial infections is essential to prevent economic losses in fish farms. The protocols developed here will provide functional and reliable tool to monitor fish vulnerability to this pathogen and to study '*ex vivo*' the benefits of functional feeds. In addition, gilthead seabream skin mucus could represent a potential source of new therapeutic compounds for treating biofilms of this pathogen, due to its effect on established biofilms.

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# SCALING UP AEROBIC SLUDGE REDUCTION FOR END-OF-PIPE TREATMENT OF RAS SLUDGE OR NUTRIENT MOBILISATION FOR INTEGRATED AQUACULTURE SYSTEMS

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## Introduction

Aquaculture systems generate sludge continuously and inevitably. This sludge consists of indigestible feed components, uneaten feed, and microbial biomass and contains high amounts of nutrients. It can thus be treated as valuable side stream under the circular economy paradigm, being useful for e.g., aquaponics to reduce reliance on inorganic fertilizer. In any case, it must be regarded as a residual material whose disposal entails costs. Within this context, aerobic sludge stabilization is a fast and simple process that offers high nutrient recovery efficiency and nitrogen availability. While the process is more energy-intensive than anaerobic digestion (Chen et al., 1997), it offers the advantage of a lower hydraulic retention time (HRT), making it potentially more cost-effective as bioreactors of large volume require high additional investment. However, there are no studies that assessed the optimization potential and the economic side of aerobic sludge stabilization in aquaculture, let it be as a means of end-of-pipe treatment or a method for nutrient mobilization, targeting aquaponic systems.

Against the outlined background, this study aimed at optimizing aerobic sludge stabilization by investigating the influence of pH, temperature, duration, and presence of PSB on the reduction of organic matter and nutrient leaching. The obtained results were then validated on pilot-scale to assess scale-effects.

## Material and Methods

A series of experiments was conducted to identify the maximum solids load, hydraulic retention time, and the effect of PSB and commercial enzymatic products for sludge treatment. Sludge for the experiments originated from the drum filter backwash of a RAS stocked with *Clarias gariepinus* and fed with Skretting Meerval throughout sludge collection. 1 L glass bottles equipped with a cap and aerated through PVC pipes served as bioreactors. The bottles were placed in a water bath with temperature regulation.

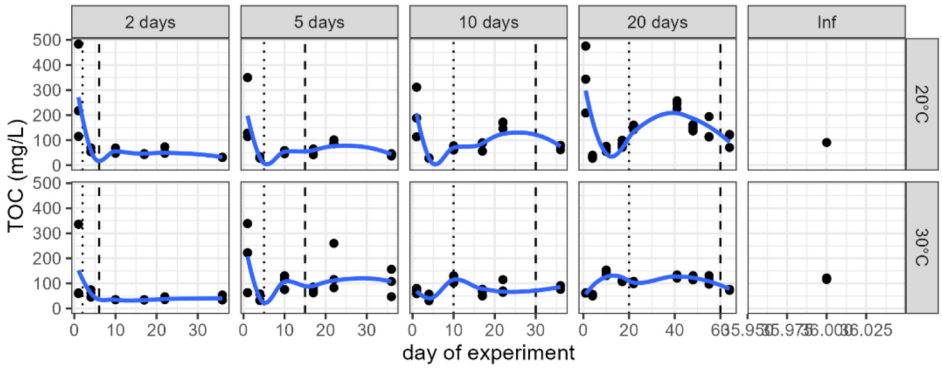


Figure 1: Total Organic Carbon (TOC) concentration in continuous aerobic sludge reduction reactors (n = 3) operated at different hydraulic retention times (HRT). Blue line: Moving average. Dotted line: 1x HRT. Dashed line: 3x HRT. Batch reactor (HRT = inf.) was used as control.

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The maximum solids load was determined by mixing sludge with a known amount of total solids (TS) with deion. $H_2O$ to achieve TS loads from 5 to 25 mg $L^{-1}$. The reactors were then aerated over seven days and the oxygen saturation determined daily. Eventually, the effect of the maximum solids load on sludge reduction performance with and without addition of PSB was assessed in batch experiments. The respective amount of TS was added and the reactor kept under continuous aeration over 14 days.

The optimum hydraulic retention time (HRT) for continuous sludge reduction was determined at two different temperatures (20°C and 30°C) and four HRT levels (2 d, 5 d, 10 d, 20 d), with a batch treatment as control. Water exchanges were carried out daily with exchange water set to the pre-determined maximum TS concentration. Experiments were run until at least 3 times HRT was reached.

Abiotic parameters were determined daily in the batch experiments and in increasing time intervals in the continuous experiment. Water and sludge samples for C, N, and P analyses were analysed according to standard methods.

Results and Discussion

The maximum TS load under which the reactor remained aerobic was 10 g $L^{-1}$. This is five to ten times lower than the loads reported in aquaponics literature (Goddek et al., 2018; Monsees et al., 2017). As expected, temperature had a significant effect on the remineralization process in aquaponic sludge treatment. In batch experiments, the pH reached its minimum after approx. 6 days at 30°C, compared to 14 days at 20°C, indicating accelerated microbial activity. Similarly, the degradation of total organic carbon (TOC) showed a decline around day 4–5 across most treatments, except at higher HRT (10 and 20 d) at 30°C. While TOC concentrations were lowest (~50 mg $L^{-1}$) in treatments with shortest HRT, they increased with longer HRT, ranging from 75 to 150 mg $L^{-1}$. Total nitrogen concentrations in solution increased over time, reaching their inflection point faster at 30°C (~7 days) compared to 20°C (~14 days). Higher nitrogen concentrations were associated with longer HRT and warmer conditions. A 5-day HRT appears optimal, balancing pH reduction and system feasibility. At this duration, pH dropped to 5.2 at 20°C and 4.5 at 30°C, which is sufficient to mobilize plant nutrients due to shifts in speciation.

In practical terms, a small recirculating aquaculture system (RAS) with a total volume of 5 $m^3$ and a daily sludge production of about 3 L/d (50 g/L DM) would require a 75 L tank (based on the 5-day HRT and 10 g/L TS target) for sludge stabilization. However, an improved solid-liquid separation is necessary to achieve optimum performance.

Acknowledgements

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# USE OF ARMS-MBON TECHNOLOGY FOR ENVIRONMENTAL MONITORING OF AREAS AFFECTED BY LOCAL IMPACTS: CASE STUDY IN PORTS OF THE GULF OF VALENCIA

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**Introduction**

Monitoring benthic biodiversity in harbours and human-impacted areas is essential for assessing environmental status, detecting changes due to pollution, and managing ecological risks associated with anthropogenic activities.

Benthic communities are key indicators of environmental health, as they respond sensitively to pollutants and shift in their composition and diversity may signal environmental degradation *(Chatzinikolaou et al*., 2018).

Continuous monitoring helps to quickly detect the introduction of harmful species, allowing effective responses to prevent their spread and minimize ecological and economic damage. Degraded environments facilitate the spread of unwanted species and are associated with an increase in invasive species, which can affect ecosystem functionality (Bayley *et al.*, 2024; Chatzinikolaou *et al*., 2021; Tamburini *et al*., 2020)they can affect biodiversity, industry, ecosystem function, and both human and wildlife health. Isolated areas with sensitive or highly specialised endemic species can be particularly impacted. The global increase in the scope of tourism and other human activities, together with a rapidly changing climate, now put these remote ecosystems under threat. In this context, we analyse invasion pathways into South Georgia and the South Sandwich Islands (SGSSI.

For this reason, the development of standardised accessible methodologies for environmental monitoring of biodiversity, such as ARMS-MBON (Autonomous Reef Monitoring Structures of the Marine Biodiversity Observation Network), is crucial, because they allow more accurate, rapid and large-scale data collection on biological diversity, overcoming many limitations of traditional methods (Daraghmeh *et al*., 2025; Obst *et al.*, 2020)we present the data of our first sampling campaign comprising 56 ARMS units deployed in 2018–2019 and retrieved in 2018–2020 across 15 observatories along the coasts of Europe and adjacent regions. We describe the open-access data set (image, genetic and metadata.

The present study aims to use these standardised environmental monitoring methodologies to observe variations in biodiversity among different types of ports in the Gulf of Valencia (marina, fishing and international cargo) and evaluate their applicability to study other types of marine areas affected by anthropogenic activities.

**Material y methods**

Three ports located in the Gulf of Valencia (Spain) are being studied: Pobla de Farnals (marina), Denia (fishing port), and Valencia (international cargo port).

Three ARMS have been placed in each of these ports, where they will remain submerged for around three months (July 2025-October 2025). Once the immersion time has elapsed, they will be extracted and processed in the laboratory as specified in the methodology proposed by Obst *et al*. (2020), to extract the surrounding biological material.

Subsequently, molecular genetic analyses will be carried out on the material scraped from the ARMS plates, which will be compared with DNA databases (COI, 18S and 16S metabarcoding).

**Expected results**

The results of the molecular genetic analysis of each of the ARMS are expected to enable the characterisation of the biological communities in each port and their differences. These are predicted to focus on indicator species of different levels of pollution, species typical of enclosed waters, and even non-indigenous species that may have been brought in by different types of marine traffic. It will also be possible to evaluate the performance of this methodology for monitoring areas of direct anthropogenic impact, such as ports or aquaculture farms.

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**Future lines**

One of the objectives of the study is to draw up a future strategy for monitoring and characterising local impacts and biodiversity hot-spots. For this reason, we are testing this methodology in confined environments such as ports. Afterwards, we would apply the same methodology to monitor the impact of aquaculture facilities, evaluate the effect of marine nature reserves, or control the biological diversity of areas with special characteristics for the conservation of endangered species.

**Acknowledgements**

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# FIRST STEPS FOR A LONG TERM ENVIRONMENTAL AND COASTAL BIOCENOSIS MONITORING PROTOCOL IN THE VALENCIAN COMMUNITY, SPAIN (W MEDITERRANEAN SEA)

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**Introduction**

The Mediterranean Sea is suffering from multiple pressures affecting severely its biodiversity. The region is warming faster than any other sea, causing marine heat waves and ecosystem degradation (Klaoudatos et al., 2024). Phenomena such as 'tropicalisation' and 'meridionalisation' are altering the distribution of species, benefiting warm species and displacing native ones. Corals and gorgonians forests are suffering from bleaching and mortality (Ghanem et al., 2021; Kersting et al., 2023). In Spain, the Plan Complementario de I+D+I en Ciencias Marinas ThinkInAzul (Government of Spain, 2012) has been promoted and one of its objectives is the monitoring of the marine environment. Fish monitoring is crucial, and methods such as the Underwater Visual Census (UVC) have been improved (Azzurro et al., 2022), but it is necessary to standardize the use of sustainable techniques to assess the health of the marine ecosystem.

**Materials and methods**

A protocol for monitoring impacts of climate change was standardized by Izquierdo-Muñoz, et al. (2024) and has been implemented for 2-years monitoring of benthic biocenosis and singular species in 4 stations (Oropesa, Pobla de Farnals, Albufera and Calp) along the coast of the Valencian Community, Western Mediterranean Sea. The monitoring protocol was established by conducting Underwater Visual Census (UVC) at rocky bottom locations, coupled with sensible sessile species monitoring (e.g., hard and soft corals) covering a 250 $m^2$ of sampled area in each census under the criteria of presence of the species selected of interest by ThinkInAzul.

**Results**

A total of 4887 specimens were sighted in the UVC surveys. Differences in the fish composition of the rocky bottom communities show significant differences in relation to season of the year.

Environmental deterioration recorded in the 2084 coral colonies observed in the surveys show mostly healthy specimens with little deterioration. Seasonal variations in the colonies examined showed not significant differences for most of the sample areas.

**Discussion and conclusion**

The protocol for monitoring impacts of climate change has been applied for a period of 2-years to establish a baseline for comparison in future surveys. The results show indicators of resilience so far to the impacts of marine warming as observed in the low levels of coral bleaching and the dominance of *Coris julis* over *Thalassoma pavo*. A shift in proportions between the two species is expected, reducing the abundance of *C. julis* and increasing *T. pavo*, linked to the increase in temperature (Azzurro et al., 2022). Future surveys will provide an early warning system thanks this bioindicators that will allow the detection of climate change trends and its adaptation to other areas of the Mediterranean, with variation and criteria and community species. It is therefore essential to continue the challenges of ThinkInAzul to understand the trends of change in the Mediterranean Sea.

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# EFFECTS OF TEMPERATURE AND SALINITY ON THE GROWTH OF EARLY LIFE STAGES OF THE JELLYFISH *Pelagia noctiluca*: OPTIMIZING AQUACULTURE TECHNIQUES FOR SUSTAINABLE CULTIVATION

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## Introduction

Jellyfish have been recognized as valuable sources of novel natural products with a wide range of biotechnological applications. However, the spatiotemporal variability of their life cycles makes them an occasional resource, whereas aquaculture can offer a continuous and reliable supply. *Pelagia noctiluca* is an ideal candidate for research in the biomedical, cosmetic, and pharmaceutical industries due to the potential value of bioactive compounds found in its venom (Frazão et al., 2017). Despite its importance in marine biotechnology, the cultivation of *P. noctiluca* is considered a challenge by the scientific community, aquariums, and industry. This is primarily due to its holoplanktonic life cycle (Canepa et al., 2014), which lacks a polyp stage—an element that, in jellyfish with metagenetic life cycles, serves as an inexhaustible source of young jellyfish (ephyrae and metaephyrae). In this context, this study aimed, for the first time, to investigate the effects of temperature and salinity on the growth of early life stages of *P. noctiluca*, with the goal of optimizing aquaculture techniques.

## Materials and methods

Fertilized eggs from mature *P. noctiluc*a medusae at Loro Parque Aquarium (Tenerife, Spain) were incubated in filtered seawater with aeration until they reached the ephyra stage on day 3 post-fecundation. Experiments were conducted in 18 containers combining two temperatures (18 °C, 24 °C) and three salinities (32, 36, 40 PSU), with three replicates per treatment. The initial number of ephyrae per culture was 30, and they were acclimated using a drip method. Ephyrae and metaephyrae were fed daily with 20,000 rotifers and containers were cleaned twice weekly to maintain water quality. Morphological descriptions and the total body diameter (TBD) of 6 live individuals per treatment and replicate were recorded weekly (Ballesteros et al., 2022a). TBD values were converted into carbon weight (CW) to calculate growth rates (GR) according to Lilley et al. (2014) and, life cycle stages were classified according to Ballesteros et al. (2021). A two-way ANOVA assessed the effects of temperature and salinity on GR, with post hoc Tukey's tests applied ($p < 0.05$). The experiments were finished at day 24 post-fecundation.

## Results

A two-way ANOVA revealed significant effects of temperature, salinity, and their interaction on GR ($p < 0.01$). The highest GR was observed at 24 °C/32 PSU ($0.23 \pm 0.03$ d$^{-1}$) where jellyfish exhibited fully developed marginal tentacles and a high number of nematocyst batteries by day 24 post-fecundation (mean TBD of $1.42 \pm 0.22$ mm). At 36 PSU, GR at 24 °C ($0.21 \pm 0.03$ d$^{-1}$) was significantly higher than at 18 °C ($0.14 \pm 0.03$ d$^{-1}$), highlighting the positive effect of increased temperature at moderate salinity. However, individuals from both treatments were classified as metaephyra stage 3, with mean TBDs of $1.25 \pm 0.16$ mm and $0.78 \pm 0.13$ mm, respectively, at day 24 post-fecundation. Finally, jellyfish in the 18 °C/40 PSU and 24 °C/40 PSU cultures did not show tentacle buds and were classified as ephyra stage 2 with mean TBD values of $0.65 \pm 0.12$ mm and $0.77 \pm 0,19$ mm at day 24 post-fecundation, respectively.

## Discussion

Despite the importance of *P. noctiluca*, few studies have focused on optimizing its life cycle in aquaculture (Lilley et al., 2014; Ramondenc et al., 2019; Ballesteros et al., 2022a, 2022b)causing considerable problems to tourism and aquaculture. Little is understood about the drivers of its periodic presence and absence or how it survives unfavourable periods. Studying the effect of starvation, we evaluated the main metabolic expenses (reproduction, respiration and excretion, and the combined effects of temperature and salinity have never been investigated at any stage of its life cycle with the specific aim of improving aquaculture practices. Previous captivity studies identified 18°C the optimal temperature for the culture of *P. noctiluca* (Lilley et al., 2014;

Ramondenc et al., 2019)causing considerable problems to tourism and aquaculture. Little is understood about the drivers of its periodic presence and absence or how it survives unfavourable periods. Studying the effect of starvation, we evaluated the main metabolic expenses (reproduction, respiration and excretion, and indicated that higher temperatures induced stress in the individuals. However, Ballesteros et al. (2022a) recommended a temperature of 22 °C as more suitable for promoting healthy growth without inducing visible signs of stress in captivity. In this study, we demonstrated that temperature and salinity had a significant effect on the growth of *P. noctiluca* ($p < 0.01$), with 24 °C and 32 PSU identified as the optimal combination for proper development. Cultivating the species at high salinities, such as 40 PSU, is not recommended, as it does not exhibit proper growth under these conditions, regardless of temperature. These guidelines, together with dietary recommendations for the species (Ballesteros et al., 2022a) and the implementation of new breeding systems (Ballesteros et al., 2022b), represent a significant advance in the development of cultivation protocols for the mauve stinger *P. noctiluca*.

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# XVIII-CENTURY TSUNAMI IN THE ALBUFERA DE VALENCIA (SE SPAIN)

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**Introduction**

The Albufera is to the south of the city of Valencia, (Fig. 1), which is currently a coastal lake of fresh to brackish water (Sanchis Ibor, 2010) regulated by three systems of watergates called the Pujol, Perellonet and Perelló (from north to south). At the beginning of the 17th century, Escolano (1611) described the Albufera with a single water channel connecting with the sea, the Albufera Gola, which was different from the current ones, near Casa del Rey (the current Rey Beach, Sanchis Ibor, 2010). It was wide enough to admit salt water, and was artificially closed with logs, branches, and sand so that the Albufera could function as a large fish farm that supplied abundant and constant fish to the city of Valencia. By closing off the communication with the sea, the level of the lagoon could vary with respect to sea level. A mark was required to monitor the water level in the Albufera, and this corresponded to the so-called "Fita," which translates as "The Milestone." Although it seems that one existed before, king Jaume I rebuilt it (it was in place by 1250), marking it with his royal coat of arms (Sanchis Ibor, 2010). The Fita had marks located at a distance of Valencian palms (approximately 22.5 cm). When the Albufera was connected to the sea, the Fita protruded one palm, and when the level of the Albufera dropped during the summer, it could drop three palm marks (66 cm). If the water level dropped further, in ancient times the channel had to be opened (Sanchis Ibor, 2010) or the Albufera had to be filled with waters from the Turia and Jucar rivers. This way, the fishing was not lost. If the level was higher than the "Fita", the dam that closed the inlet could break, losing all the possible fishing, so when the level rose, the dam must be opened in a controlled manner to capture the fish.

The Fita's position is only marked on a map by Pedro Torbé (commissioned by the Crown to carry out a new boundary marking of the Albufera) from 1752. Its original location is marked in the Fishermen and Farmers Society of Sueca maps by Toni Carrasquer (1984). Around 1760, the original Fita with the royal coat of arms was missing, and the mention of "Fita" disappears from maps and archives (Sanchis Ibor, 2010).

The maps by Toni Carrasquer García (1984) show the position of the Creu de la Llonga, which was found as scattered ruins on a terrace. It has appeared in the area since the 18th century, which coincides with the destruction/relocation of the "Fita" of the Albufera as deduced from the Torbé map (1752). The Creu de la Llonga is identified as the Fita with the palm marks. The place name *la Llonga* does not appear on the Torbé map (1752) but it does appear on the recent maps.

**Discussion and conclusions**

Between 1755 and 1756, two tsunamis occurred in the Mediterranean: on November 30, 1755, the Lisbon earthquake tsunami affected the western Alboran Sea (Silva et al., 2019), and on January 31, 1756, the Santanyí tsunami in Mallorca (Roig-Munar et al., 2023). For Roig-Munar et al. (2023), the tsunami blocks in Mallorca seem to have shifted around the late 18th and early 19th centuries, with the Santanyí tsunami being the main candidate. Roselló Verger (1979) described several blocks of a cemented Tyrrhenian dune in the Perellonet area of the Albufera sandbank and these could be the product of the mid-18th-century event.

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The original position of the “Fita” in the Albufera, taken from the Toni Carrasquer García maps (1984), and the current position of the Creu de la Llonga are 4,300 m apart in a heading of approximately N250°E.

The old “Fita” of the Albufera, which was used to control the lake level when it was connected to the Mediterranean Sea, is identified as the Creu de la Llonga. It is proposed that its displacement may have been caused by a tsunami like the one that hit Santanyí in Mallorca in 1756. This event may also have displaced blocks from the underwater sandbank to the beaches of La Dehesa.

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# POTENTIAL AND CHALLENGES OF ALGAE BASED FUNCTIONAL DIETS FOR ENHANCING SEABREAM RESILIENCE

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## Introduction

Functional diets play a pivotal role in aquaculture by enhancing fish health, resilience, and performance under the various stressors encountered throughout the production cycle, such as handling, transport, or high stocking densities. The integration of functional additives (e.g. prebiotics, probiotics, amino acids, and bioactive compounds from micro- and macroalgae) into aquafeeds has shown potential to mitigate oxidative stress and support immune responses. Among these additives, algae-derived compounds have gained particular attention for their richness in natural pigments, antioxidants, and polysaccharides. These ingredients may contribute to health status and help maintain gut integrity, which is essential for nutrient absorption and overall resilience. Species such as gilthead seabream (*Sparus aurata*), a key species in Southern European aquaculture, can particularly benefit from these dietary strategies. However, the successful implementation of functional diets requires a rigorous process of fine-tuning through controlled trials to determine optimal formulations and dosages. These trials are essential to ensure efficacy, cost-effectiveness, and compatibility with existing farming practices, while supporting the development of a range of reliable and sustainable aquafeed products for the market. As part of this effort, the Vertical Algae project aims to develop algae-derived products for a range of applications. Specifically, sub-project 5 is dedicated to aquaculture, focusing on the formulation of functional diets that incorporate algal biomass or extracts, with the goal of enhancing fish resilience through improved nutritional support.

## Materials and Methods

**Trial 1:** A 3-week feeding trial was conducted to evaluate the effect of microalgal extracts on the resilience of juvenile gilthead seabream (± 18 g) to stress and bacterial challenge. Fish were fed one of three diets: a commercial-like premium control diet supplemented with immunostimulants (CTRL+), or the same diet further supplemented with either a low (LOW) or high (HIGH) dose of *Tetraselmis chui* extract. At the end of the feeding period, fish were sampled to assess physiological and molecular biomarkers related to antioxidant status, gut health, and immune function. Subsequently, fish were exposed to an acute stress challenge involving handling, crowding, and transport. Six hours after the onset of the stressor, additional samples were collected to evaluate the fish response to the challenge. Following this, fish were infected with *Photobacterium damselae* subsp. *piscicida*, and mortality was monitored for 10 days.

**Trial 2:** In a second trial, early juvenile seabream (± 6.5 g) were fed for 7 days. The dietary treatments included the same commercial-like premium diet used in Trial 1 (CTRL+), a control diet without immunostimulants (CTRL−), and two additional diets based on the CTRL− formulation, supplemented with a low dose of *Tetraselmis chui* extract combined with either *Ulva* extract (Low+UL) or processed *Euglena* biomass (Low+EU). At the end of the feeding period, fish were sampled as before. Afterwards, they were subjected to the same stress protocol, followed by infection with *P. damselae* subsp. *piscicida*. Additional samples were taken 6 hours post-infection to assess the early fish response to this challenge, and mortality was monitored over the subsequent 10 days.

## Results and Discussion

**Trial 1:** Following the stress challenge, fish fed the LOW diet exhibited significantly higher hepatic total antioxidant status (TAS) than those fed CTRL+ or HIGH, suggesting a protective effect of the lower *Tetraselmis* dose against oxidative stress. Both LOW and HIGH diets appeared to buffer oxidative stress effectively, as lipid peroxidation (LPO) levels remained stable post-stress, unlike in the CTRL+ group, which showed a significant increase. Despite these physiological effects, no significant differences in mortality were observed between groups following the *P. damselae* subsp. *piscicida* challenge.

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**Trial 2:** Following the stress and bacterial challenge, liver catalase (CAT) activity was significantly higher in fish fed the LOW+UL and LOW+EU diets compared to the CTRL− but not the CTRL+ groups, indicating enhanced antioxidant responses with algal supplementation. In the intestine, fish fed the LOW+EU diet showed upregulated expression of *il1b* and *cldn12* compared to those fed the CTRL+ diet. The elevated expression of *il1b*, a key pro-inflammatory cytokine, suggests an activated local immune response, potentially modulated by the algal supplementation. Simultaneous upregulation of *cldn12*, a tight junction protein, may reflect a strengthening of the gut barrier, which is critical during infection. These findings point to a potential synergistic effect between *Tetraselmis* and *Euglena* in supporting intestinal immune responses and maintaining barrier integrity under stress and pathogen challenge. As in Trial 1, no differences in mortality were observed between treatment groups during the post-infection period.

**Conclusion**

Both trials highlight the potential and the challenges of using functional diets to improve fish resilience. While some algal extracts showed positive effects on antioxidant status and gut health, these changes did not lead to improved survival after bacterial infection. Functional diets can produce specific effects in different tissues, and results often depend on context, making it difficult to rely on single biomarkers. This reinforces the need for broad datasets and multivariate analyses to fully understand the impact of these diets. In the end, developing effective functional feeds requires a careful, data-driven approach that considers species, ingredients, and farming conditions.

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# DETERMINATION OF THE TEMPERATURE MINIMUM FOR FEED INTAKE IN COMMON CARP

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## Introduction

The global climate of our planet is undergoing a rapid warming phase, characterized by a sustained increase in atmospheric and surface water temperatures The annual mean temperature is continuously rising also in Hungary, resulting in a surface water temperature increase of 0.32°C by average per decade over the past four decades. This warming is particularly significant in shallow lakes. The increase of temperature in the winter months is almost twice as high as in the summer (Li Huan et al., 2024).

Climate change is increasingly affecting pond aquaculture as well, with challenges including rising and fluctuating water temperatures. Additional emerging risk factor is higher water temperature during overwintering with associated metabolic impacts, and the emergence of various infections. These direct and indirect stressors negatively affect the welfare and immune systems of traditionally farmed species, influencing fish viability and production outcomes. The digestion capacity and appetite are primarily temperature-dependent in case of our most important farmed fish species common carp (*Cyprinus carpio*). In winter, natural food disappears in the lakes, but fish show active periods following weather fluctuations. Recent studies have shown measurable activity even at temperatures below 4°C. (Bauer & Schlott, 2004). Some studies suggest that carp not only moves but actively feeds at 6°C, with increasing feed intake as temperatures rise (Chen Song-bo et al., 2012). Active feeding behaviour typically occurs during milder winter periods, during which nutrient supply can only be managed through feeding. The primary goal during this period is to maintain condition and minimize weight loss, which is essential for sustaining a healthy stock in spring. Under these conditions, we aimed to determine feed intake at low temperatures, with a particular focus on identifying the lowest temperature at which the fish stop feeding. Our experiment focuses on reducing the vulnerability of carp farming technology to external stress factors.

## Materials and Methods

Four 160 L aquaria were used in the experiment: three with a feeding regime and one control without feeding. Each aquarium was stocked with five one-summer-old carp (average weight: 89.5 ± 23.9 g). The aquariums were filled with aerated tap water, with salt (NaCl) added at a concentration of 4 mg/L to prevent the spread of white spot disease (*Ichthyophthirius multifiliis*). Filtration was provided, with integrated heating, and adequate oxygen levels were maintained using an air pump.

The acclimation period lasted 17 days, during which water temperature fluctuated between 6–7°C. After acclimatization, measures were taken (standard and total length, body weight) of each fish. The experiment started at 4°C (3.4–4.4°C) and was maintained for 10 days using natural atmospheric cooling. For the subsequent temperature ranges (6°C and 8°C), the heaters were used to achieve the desired temperatures. Water temperature and oxygen levels were measured daily. Feeding was carried out once a day using Aller Master (Aller Aqua A/S Denmark) 4.5 mm feed pellets. Uneaten pellets were collected and counted after 24 hours, because of the pellets' good water stability and distinctive shape and size, which differed sufficiently from fish faces, allowed accurate counting. The trial lasted totally 56 days, when the fish biometric indices were assessed.
Feed intake was calculated using the following formula:
Feed provided (pcs) – uneaten feed (pcs) = feed consumed (pcs)
Feed consumed (pcs) * unit weight of the pellet (g) = feed consumed (g)
Feed consumed (g) / total fish biomass (g) = feed intake (%)
The average oxygen level measured during the experiment was 89.7 ± 9.3%.

*(Continued on next page)*

**Results and Discussion**

The fish were inactive at 4°C with minimal feed intake (0.031%). With increasing temperatures to 6°C, feed intake increased to 0.3% in all three aquaria. After two days, feed consumption decreased, presumably due to satiety, followed by a slower, steadier increase in feeding up to 0.160%. By further increasing the temperature to 8°C, feed intake reached 0.431%. Feed consumption showed significant differences between temperature levels (4–6–8°C) and a strong positive correlation was observed between water temperature and feed intake.

A slight increase in body weight was observed in fed fish across the temperature ranges. The initial average weight of 81.4 ± 23.5 g increased to 82.5 ± 25.4 g after eight weeks (56 days) treatment period, while the unfed control group showed decrease from 100.0 ± 28.7 g to 94.7 ± 27.4 g. As the temperature was higher the body weight loss of unfed fish was higher.

**Conclusion**

We demonstrated that at 4°C, the fish either did not consume feed or fed only minimally. However, feed intake was observed at 6°C and became more pronounced at 8°C, showing a strong positive correlation with increasing water temperature. Additionally, a slight increase in fish body weight was observed. Overall, the results indicate that targeted winter feeding can reduce body weight loss during overwintering, resulting better condition of the fish, which may enhance disease resistance and improve production efficiency.

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# EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS ON GROWTH, PHOTOSYNTHESIS AND ANTIOXIDANT ACTIVITY IN MARINE MICROALGAE

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## Introduction

The increasing use of technologies that emit extremely low frequency electromagnetic fields (ELF-EMF), such as submarine cables and electronic tagging systems, raises concerns about their potential ecological effects in marine ecosystems. Although the biological impacts of ELF-EMF on humans have been widely studied, their effects on marine primary producers, especially microalgae, remain poorly understood. Microalgae play essential roles in aquatic ecosystems and can synthesize protective molecules such as carotenoids and phenolic compounds in response to stress. This study evaluates the effects of ELF-EMF exposure on growth, photosynthetic performance, and antioxidant activity in marine microalgae strains from both anthropized and pristine environments.

## Materials and Methods

Six strains of microalgae from four taxonomic classes (Cyanobacteria, Porphyridiophyceae, Bacillariophyceae, and Chlorophyceae) were cultivated in liquid medium and exposed for five days to ELF-EMF (50 Hz, 100 ± 20 μT) generated by Helmholtz coils. Cultures were maintained under controlled temperature and light conditions. Cell density was monitored daily using a Neubauer chamber, and trypan blue was added to assess cell viability. Total antioxidant capacity was measured using the Sigma-Aldrich® MAK334 kit, and photosynthetic performance was assessed with a LiCor 6800 system by measuring net $CO_2$ assimilation, electron transport rate, PSII quantum yield, and dissolved carbon. Strains were grouped based on their collection sites: the anthropized Mar Menor lagoon (acclimated group) and the minimally impacted Moratalla mountains (non-acclimated group).

## Results and Conclusion

No significant differences in cell density (Figure 1) or total antioxidant capacity were observed between control and ELF-EMF-treated groups. However, growth trends varied by strain, with slightly higher final densities in controls. Photosynthetic responses showed significant interspecies differences: Tetraselmis sp. exhibited altered $CO_2$ assimilation and electron flow; Erythrolobus sp. and Scenedesmus sp. showed significant changes in dissolved carbon, and the latter also in PSII quantum yield. Acclimated strains tended to respond differently than non-acclimated ones in antioxidant activity, suggesting possible adaptive responses. Overall, short-term ELF-EMF exposure did not cause strong physiological changes in the strains tested, but species-specific photosynthetic responses and environmental history may modulate sensitivity. Further long-term studies are recommended to assess cumulative or delayed effects.

# ESTABLISHMENT OF IDENTIFICATION METHOD OF SEA CUCUMBER SPECIES TRADED IN KOREAN MARKET USING MULTIPLEX PCR AND PEPTIDE-NUCLEIC ACID (PNA) BASED REAL-TIME PCR METHOD

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## Introduction

The expansion of Free Trade Agreements (FTAs) has significantly increased seafood imports, raising concerns about mislabeling of country of origin and consumer safety. Sea cucumbers are imported in substantial quantities, exceeding 1,000 tons annually, in various forms such as dried, frozen, salted, or preserved in brine. The emergence of incidents involving origin fraud underscores the necessity for accurate and efficient molecular identification tools. This study aimed to develop and validate multiplex PCR and peptide nucleic acid (PNA)-based real-time PCR assays for species-level identification of imported and distributed sea cucumbers in the domestic market.

## Materials and methods

A total of 32 sea cucumber samples were collected from various retail sources, including domestic supermarkets, traditional markets, and online shopping platforms. Species identification was carried out using small subunit ribosomal RNA (SSU rRNA) gene sequencing, which identified four species: *Cucumaria miniata* (Red sea cucumber), *Stichopus horrens* (Ruby cucumber), *Apostichopus japonicus* (Japanese sea cucumber), and *Holothuria spinifera* (Brown sea cucumber). Based on these results, four species-specific primer pairs were designed for multiplex PCR, along with four corresponding PNA probes for real-time PCR analysis.

## Results and discussion

The developed multiplex PCR assay demonstrated high specificity, with each primer set amplifying only the target species without cross-reactivity. The detection sensitivity ranged from $1 \times 10^3$ to $1 \times 10^4$ copies/µL. Similarly, the PNA-based real-time PCR assay showed specific amplification with distinct Tm values for each species, also within the sensitivity range of $1 \times 10^3$ to $1 \times 10^4$ copies/µL. These findings suggest that both methods are robust and reliable for species identification. The assays developed in this study are expected to provide effective tools for the authentication of sea cucumber species and for preventing country-of-origin mislabeling in the seafood market.

# CONSUMER AND PRODUCER PREFERENCES FOR ORGANIC AQUACULTURE PRODUCTS: THE CASE OF GREECE

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## Introduction

Organic aquaculture has witnessed notable expansion and gained increasing scholarly and commercial interest, largely driven by escalating consumer demand for sustainably and organically certified aquaculture products. (Lembo and Mente, 2019). In the European Union, despite the growing demand for organic aquaculture products, evidence suggests a deceleration in the sector's growth, varying across countries and species. As environmental concerns and preferences for sustainable food sources increasingly influence consumer behavior, it becomes essential to understand consumer perceptions and purchasing patterns. Of particular importance is consumers' willingness to pay (WTP) for organic aquaculture products, as it serves as a critical incentive for producers to adopt organic farming practices—practices that typically incur higher production costs than conventional methods.

Previous research conducted in Greece indicates that younger consumers with higher levels of education and income tend to be more aware of the benefits associated with organically farmed fish and seafood (Polymeros et al., 2014; 2015).

This study examines both the WTP among Greek consumers and the willingness of Greek producers to engage in the organic cultivation of seabream, seabass, and mussels.

## Materials and Methods

This study utilizes data collected from structured surveys administered to both consumers and producers. Employing contingent valuation methodology, the research assesses consumers' willingness to pay (WTP) for seafood products derived from three distinct production systems: conventional aquaculture, organic aquaculture, and wild capture. The analysis investigates the influence of consumers' knowledge of organic aquaculture practices, their environmental attitudes, and their awareness of ecological issues on their purchasing preferences. Parallel to the consumer analysis, the study also examines producers' willingness to engage in the production of seabream, seabass, and mussels under conventional and organic prodaction system, aiming to identify potential alignment or divergence between consumer demand and producer intent.

## Results and Discussion

Altintzoglou et al. (2025) found that consumer awareness of aquaculture production systems remains generally low among European populations, despite widespread favourable attitudes toward sustainability in aquaculture. Their findings suggest the presence of a "halo effect," whereby positive perceptions of organic food in general may be extending to organic aquaculture products, leading to increased acceptance despite limited knowledge. The present study adds to this discourse by offering a focused analysis of the Greek seafood market, elucidating key demand-side dynamics. The results provide insights for stakeholders—particularly producers, retailers, and policymakers—who are aiming to foster the development and adoption of sustainable organic aquaculture practices in Greece.

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# PHOTOPERIOD-RESPONSIVE AND SEX-SPECIFIC CIRCADIAN TRANSCRIPTOME IN SEA URCHIN GONADS: NOVEL INSIGHTS FROM *Paracentrotus lividus*

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**Introduction**

The European edible sea urchin, *Paracentrotus lividus* (Lamarck, 1986) is one of the most harvested invertebrate species in the Iberian Peninsula and other European regions, due to the high market price of its gonads (Toso *et al*., 2025). Populations of *P. lividus* in the wild undergo an annual gonadal cycle. In the southern of Spain, their gonads reach their highest gonadosomatic index (GSI) from January to March, aligning with their commercial availability period (Sánchez-España *et al*., 2004). Although aquaculture of *P. lividus* began in the early 1990s, challenges persist, particularly in regulating the gonadal cycle to ensure year-round production of marketable gonads. Daily and seasonal changes in light are key signals in the synchronisation of the circadian system and in the initiation, progression, and coordination of gonadal maturation in vertebrates (Mishra & Kumar 2017). In *P. lividus*, shortening day length (autumnal equinox) boosts gametogenesis rate and trigger the first spawning event, whereas extended daylight hours reduce it (Sphigel *et al*., 2003). To investigate whether this pattern is linked to photoperiod-entrained biological rhythms, we examined the gonadal circadian transcriptome of wild and sexually mature *P. lividus* (male and female) during their reproductive period.

**Materials and methods**

Wild, sexually mature *P. lividus* of commercial size were collected in March in Aguadulce (Almeria, Spain) and maintained in 60 L tanks (25 sea urchins/tank) at the facilities of the SCI-CM (University of Cadiz). The sea urchins were kept under natural photocycle (12L:12D) and temperature (~ 15 °C), and fed *ad libitum* with a mix of ulva (70%) and kelp (30%). They were then divided into 2 groups: one kept in 12L:12D (LD group) until the sampling day, and a second group exposed to 24 h of darkness (24D) prior to the sampling day. Gonads from 4 males and 4 females were collected every 4 hours over 24-hour, immersed in liquid nitrogen and stored at -80 °C. RNA was extracted using Quick-RNA™ Miniprep Kit, purified using OneStep™ PCR Inhibitor Removal kit (Zymo Research) and sent to Novogene to obtain the transcriptome using the *P. lividus* genome as query. Analysis of rhythmicity was performed using normalized TPM as input in the DiscoRhythm web application (Carlucci *et al*., 2021) and R Studio was used for data representation and gene clustering.

**Results**

All the gonads were fully mature, containing eggs and sperm, with an average GSI of around 3 in males and females in both photocycle groups (LD and 24D). Cosinor analysis of 24,337 genes from the transcriptome revealed daily rhythmic expression in 3.8% of male genes and 4.5% of female genes under LD, with only 1.6% of these rhythmic genes being shared between sexes. These percentages increased to 9% in males and 5% in females under 24D, with only 3.8% of these genes exhibiting rhythmicity in both sexes. Comparison of LD and 24D groups showed that only 3.5% of rhythmic genes in males and 3% in females were present in both conditions. Clustering of rhythmic genes according to their peak of expression (acrophase) revealed that, in the LD group, 46% of male genes peaked around dusk, while 59% of female genes peaks at night. In males under 24D, 36% of the genes maintained their original timing of maximal expression, 59% were delayed, and 5% were advanced. In females under 24D, only 8% of genes maintained their expression peak, 51% genes were delayed and 41% were advanced. Target analysis of the canonical genes of the molecular clock only showed significant rhythmic expression of *bmal2* in females and *timeless* in males under LD condition but none under 24D.

*(Continued on next page)*

**Discussion**

The animals were healthy and had ova and sperm, with a GSI whitin the expected range for this time of year (Sánchez-España *et al.*, 2004). The genome of *P. lividus* and the transcriptome of its gonads have recently been obtained. The findings suggest variations in the transcriptome related to sex and maturation (Machado *et al*., 2022). Accordingly, cosinor and clustering analyses revealed distinct sexual differences in the diel rhythmic expression of the analysed genes. Notably, only 1.6% and 3.8% of rhythmic genes were shared between sexes under LD and 24D, respectively. Moreover, clustering analysis showed that females appear to be more nocturnal and males more crepuscular under LD. Under 24D, gene expression phases shifted in a sex-specific manner: most genes were delayed in males and a substantial proportion were advanced in females. Interestingly, the percentage of rhythmic genes in both sexes increases under 24D. These results suggest that other synchronisers, in addition to light, may also be present, but their effects could be masked by the light cues in the LD group. The lack of rhythmic expression of canonical clock genes suggests that the diel rhythms in the gonads may be driven or maintained by central or systemic signals rather than by an autonomous oscillator within the gonads. Alternatively, different genes than those found in vertebrates may be involved in the functioning of the clock in sea urchins. In summary, our findings provide novel evidence suggesting that the circadian expression rhythms of sea urchin gonads appear to be photoperiod-responsive and sex-specific. Further research is required to determine whether central/systemic cues are the main drivers of these rhythms and how they interact with the seasonal reproductive timing of sea urchins.

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# EVALUATION OF CANOLA MEAL AS A FEED INGREDIENT FOR ATLANTIC SALMON (*Salmo salar* L.)

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## Introduction

With a favourable nutritional profile and consistent supply, canola meal (CM) stands out as a promising ingredient for salmonid feeds. While several studies have examined CM inclusion in feeds for rainbow trout and Chinook salmon, its application in Atlantic salmon remains underexplored. The objective of this study is to assess the nutritional value of solvent-extracted CM by evaluating its digestibility, effects on the growth and health of Atlantic salmon, and environmental impact when used in salmon feeds.

## Materials and methods

A freshwater digestibility trial was conducted to determine the apparent digestibility coefficient (ADC) of the nutrients in CM. A reference diet was blended with CM at 90:10, 80:20, 70:30, and 60:40 ratios to create a series of test diets. Each diet was fed to triplicate groups of juvenile fish (49.0 ± 1.3 g), reared in 100-L tanks equipped with passive-feces collecting columns.

In a saltwater grow-out trial, four diets containing 0, 5, 10, or 15% CM were randomly assigned to three replicate 700-L tanks and hand-fed to post-smolt fish (245.6 ± 2.8 g) for 103 days. At the study's conclusion, a pooled sample of five fish per tank was collected for whole-body composition analysis. Blood, liver, and distal intestine samples were obtained from an additional five fish per tank to assess plasma biochemistry, liver and intestinal histology.

A life cycle assessment (LCA) following ISO and PAS 2050 standards was performed to evaluate the environmental impacts of salmon production across four scenarios: cage systems without and with 10% CM in the feed, and recirculating aquaculture systems (RAS) without and with 10% CM.

## Results

Results from the digestibility trial indicated that the ADC of nutrients was positively correlated with CM inclusion. At 40% CM, energy, protein, and essential amino acids ADC were 57.7%, 77.1%, and 83.0–93.9%, respectively. The feeding trial showed good growth performance across dietary treatments, with fish nearly tripling in body weight and maintaining a favorable feed conversion ratio (FCR) of approximately 1.0. Fish fed diets containing 5 and 10% CM had similar feed intake, growth, and FCR compared to the control, whereas 15% CM inclusion resulted in reduced growth and poorer FCR. Whole-body composition (i.e., protein, lipid, and ash) and plasma chemistry (i.e., immunoglobulin, lysozyme, glutathione peroxidase, catalase, and thyroid hormones) were similar across all diets ($P>0.05$). Intestinal and liver histology remained largely normal in all treatments. The LCA indicated that incorporating 10% CM into the diet, compared to a CM-free diet, led to 17% and 5% reduction in $CO_2$-equivalent emissions during salmon production in cage (1,683 vs. 1,390 kg $CO_2$-eq/MT salmon live weight) and RAS (5,763 vs. 5,460 kg $CO_2$-eq/MT salmon live weight) systems, respectively. Similar trends were observed for water use and land use, with impacts decreasing when CM was included. In conclusion, CM can be incorporated in salmon feed at levels up to 10% without compromising growth or health performance, while offering potential environmental benefits.

# NUTRITIONAL EVALUATION OF REDUCED ANTI-NUTRIENT FACTOR SOLVENT-EXTRACTED SOYBEAN MEAL AND MECHANICALLY PRESSED SOYBEAN CAKE IN DIETS OF ATLANTIC SALMON (*Salmo salar* L.)

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**Introduction**

In salmon diets, soy protein concentrate (SPC) is considered a safe and commonly used plant-protein ingredient, though its high cost is a drawback. In contrast, although more affordable, conventional soybean meal (cSBM) is restricted due to the presence of anti-nutritional factors (ANFs) that can reduce digestibility, impair growth, and cause gut inflammation. To overcome these challenges, a new non-GMO soybean variety with reduced ANFs has been bred and processed using two common methods: solvent extraction to produce SBM (~55% crude protein (CP) on dry matter basis) and mechanical pressing to produce soybean cake (SBC: ~53% CP). The objective of this study is to evaluate the nutritional value of SBM and SBC by assessing their digestibility, growth performance, and effects on the gut histology of Atlantic salmon.

**Materials and Methods**

In the freshwater digestibility study, juvenile Atlantic salmon (60.2 ± 0.7 g) were randomly assigned to 12 100-L tanks in groups of 25, with four replicate per group. The fish were fed with one of three diets: a reference diet and two test diets. The test diets consisted of an 85:15 blend of the reference diet and test ingredients (SBM or SBC). Fecal material was collected using the passive column method to determine the apparent digestibility coefficient (ADC) of the ingredients' nutrients.

In the saltwater feeding study, post-smolt Atlantic salmon (166 ± 2.9 g) were randomly allocated into 17 700-L tanks at 50 fish per tank. The fish were fed one of six diets: Diet A (12% SPC: positive control), Diets B and C (5% and 15% SBM), Diets D and E (5% and 15% SBC), and Diet F (15% cSBM: negative control). Each diet had three replicates, except for Diet F which had two replicates. The fish were hand-fed three times daily for 84 days. At the study's conclusion, fish body weights were recorded, and whole-body (5 fish/tank) and distal intestine samples (5 fish/tank) were collected.

**Results**

The digestibility study showed that SBM and SBC were highly digestible, with the ADC for protein and essential amino acids (EAAs) ranging from 83.8-97.2% and 78.7-96.6% for the former and latter, respectively. In the feeding trial, no significant differences were observed in growth performance or feed conversion ratio between diets. Whole-body nutrient composition and nutrient retention (e.g., moisture, protein, lipid, ash, gross energy and EAAs) were comparable across all dietary treatments. Histopathological examination showed no apparent differences between fish fed novel reduced ANFs SBM and SBC compared to those fed SPC, while improvement trends were observed when compared to those fed conventional SBM. Based on these findings, the novel soy, whether processed through solvent extraction (SBM) or mechanical pressing (SBC), is considered digestible, nutritious, and promising protein alternatives that can be included up to 15% in salmon diets.

# DEVELOPMENT OF FUNCTIONAL ANTIOXIDANT AQUACULTURE FEED INGREDIENTS FROM POULTRY WASTE VIA ENZYMATIC HYDROLYSIS

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## Introduction

The poultry industry generates large volumes of by-products such as carcasses, feet, viscera or skin, which are generally destined for low value-added purposes. However, these by-products are rich in proteins and constitute an important source for obtaining bioactive peptides of interest in animal nutrition (Fontinha et al., 2021). Through enzymatic hydrolysis, it is possible to valorise these materials by transforming them into protein hydrolysates with a high content of free amino acids and functional peptides. The aim of this work was to study the single or double enzymatic hydrolysis of chicken legs and carcasses to obtain new ingredients with antioxidant properties for aquaculture nutrition, contributing to the development of a more sustainable aquaculture and the efficient use of poultry waste.

## Materials and Methods

First, a characterisation of the sample was performed by determining its moisture, protein, fat and ash content. Chicken by-products consisting of carcasses (C) and feet (F) were diluted 1:2 with bidistilled water. Subsequently, simple hydrolysis was carried out with an endoprotease (C1, F1) for 1h at 55ºC, and sequential hydrolysis by adding an exoprotease after the action of the endoprotease (C2, F2), leaving it to act overnight at the same temperature. After that, the enzymes were inactivated by heating in a water bath (85°C, 15 min). The degree of hydrolysis of the samples was determined and then an ultrafiltration was carried out using filters with cut-offs of 30, 10 and 3kDa to study the peptide profile according to their molecular weight. The free amino acid content was determined according to Flores et al. (1997). Finally, the antioxidant activity of all extracts was measured through four different methods: ABTS radical scavenging capacity, oxygen radical absorbance capacity assay (ORAC), DPPH radical scavenging and ferric-reducing antioxidant power (FRAP).

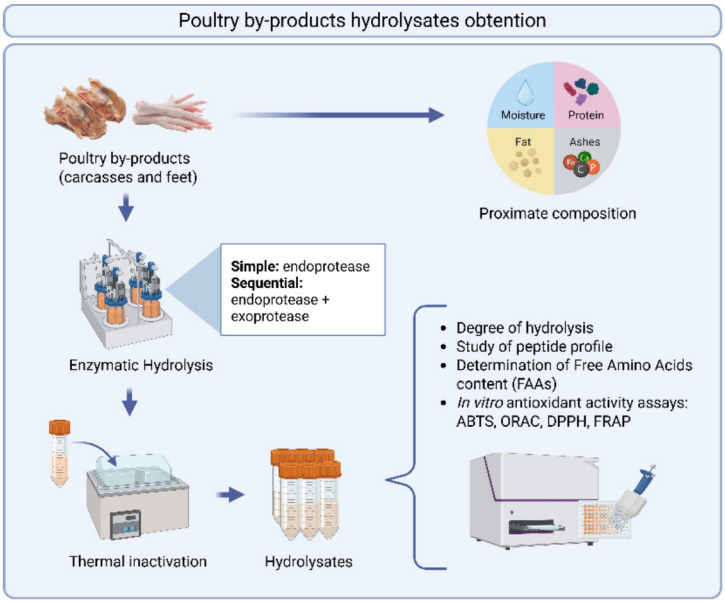


**Figure 1.** Graphical summary for the obtention of poultry by-products (carcasses and feet) hydrolysates (created in https://BioRender.com).

*(Continued on next page)*

**Results and Discussion**

Statistical analysis of the results obtained in the different trials revealed significant differences between the use of one or two enzymes to obtain hydrolysates from these by-products, as well as differences between the use of either carcasses or feet. Regarding the degree of hydrolysis, carcasses presented higher values than legs in both treatments, being hydrolysate C2 the one that reached the highest value (66.41 ± 5.47%). Peptide profile analysis showed a higher proportion of low molecular weight peptides (<3 kDa) in the hydrolysates with exoprotease incorporation (C2 and F2), with values of 78.35 ± 3.88% and 65.28 ± 2.32%, respectively. Similarly, the concentration of free amino acids was significantly higher in C2 and F2 compared to C1 and F1, with C2 standing out, where approximately 55% of the content corresponded to free amino acids. Finally, the analysis of antioxidant activity showed significant differences between carcass and leg hydrolysates, attributable to the characteristics of their peptides. Hydrolysates from chicken carcasses showed higher antioxidant activity in the ABTS and ORAC assays, while those obtained from feet were higher in the FRAP and DPPH assays. In all cases, hydrolysates obtained by sequential hydrolysis showed better results than those obtained by simple hydrolysis, which is related to a higher proportion of small peptides (< 3 kDa), widely associated with a higher antioxidant capacity (Ajibola et al., 2011).

**Conclusions**

Chicken by-products, such as feet and carcasses, can be efficiently exploited to obtain hydrolysates with bioactive properties, especially antioxidant activity. Sequential hydrolysis favoured the generation of low molecular weight peptides and a higher release of free amino acids, which resulted in higher antioxidant activity. These findings highlight the potential of revalorisation of poultry by-products through the use of enzymatic processes for the development of new functional ingredients and its application in aquaculture feeds.

**Funding**

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# GROWTH RESPONSE OF RAINBOW TROUT (*Oncorhynchus mykiss*) TO VARYING LIGHT COLOURS AND CULTURE TEMPERATURES

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## Introduction

Exogenous environmental factors, such as temperature and light, significantly influence fish growth. As poikilotherms, fish exhibit a direct correlation between temperature and metabolic rate. Light is another major factor that may affect physiology, behaviour and growth performance in indoor aquaculture systems. Specifically, variations in light wavelength and intensity can modulate metabolism and welfare status (Karakautsoli et al., 2008). This study aimed to evaluate the combined effect of light and temperature on the growth performance of rainbow trout (*Oncorhynchus mykiss*).

## Material and methods

Three different colours of light emitting diode (LED: white, 380-750 nm; green, 495-570 nm; or blue, 450-494 nm; Fig 1) were tested at two different culture temperatures (15 and 20ºC). The experimental design was 3x2 with two replicate tanks for each experimental condition. A total of 70 fish per tank, with an initial weight of 3.7 ± 0.3 g, were randomly distributed into 500 L cylindrical tanks connected to separate recirculating aquaculture systems according to temperature condition. Trout were maintained under a 12L:12D photoperiod. For 45 days, fish in all tanks were hand-fed once daily with a commercial diet to apparent satiation (two hours after the start of the light cycle) up to a maximum of 3% of daily feed intake. Fish weight was monitored every 15 days, sampling 16 fish per tank to adjust feed intake. Statistically analysis was performed using a two-way ANOVA, including the interaction effect of temperature x light. For each light condition, a Student's t-test was performed to compare temperatures. A significance level of $P < 0.05$ was considered for all tests.

## Results and discussion

There were no significant effect of light colour nor interaction with temperature on growth performance by two-way ANOVA. However, there was a significant effect of temperature by the ANOVA analysis that affected growth in a different way depending of light (evidenced when T-test was applied to compare fish weight at 15ºC and 20ºC under the same light). Fish cultured at 20ºC exhibited lower growth efficiency than those maintained at 15ºC. This growth reduction was significant for final weight (g), weight gain (WG), and specific growth rate (SGR), which decreased at 20ºC, and for feed conversion ratio (FCR), which increased, in fish exposed to green light (Fig 2). Fish under white light showed a similar trend to those under green light, though without statistically significant differences. Conversely, fish exposed to blue light maintained similar growth under both temperature conditions. Mortality remained very low (<2%) in all experimental conditions. Within an optimal range, an increase of temperature may impact on the metabolic rate, leading to increase of activity and faster feeding. In this study, all the fish ate the maximum daily feed intake stablished (3%). However, fish cultured at 20ºC showed higher activity levels during the feeding, suggesting an increase of metabolic rate. This increased energy expenditure, not compensated by additional energy intake, may have contributed to their lower growth. Other authors have demonstrated the influence of light colour on fish metabolism, stress and immune response (Chen et al., 2022). Blue light, in particular, has been shown to improve feed utilization and growth performance in other teleosts (Villamizar et al., 2009; Sierra-Flores et al., 2016) and even reduce stress levels (Wu et al., 2021). In this study, we observed that blue light could mitigate the negative effects of increased temperature on growth, compared to the reduced growth observed in fish reared under green or white light at the same feeding rate. Future results funded by this project will explore homeostatic mechanisms and welfare to draw further conclusions about how the light colour can regulate the impact of elevated temperatures in indoor aquaculture systems.

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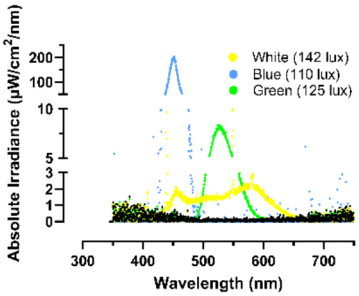


Fig 1. Light of the tanks

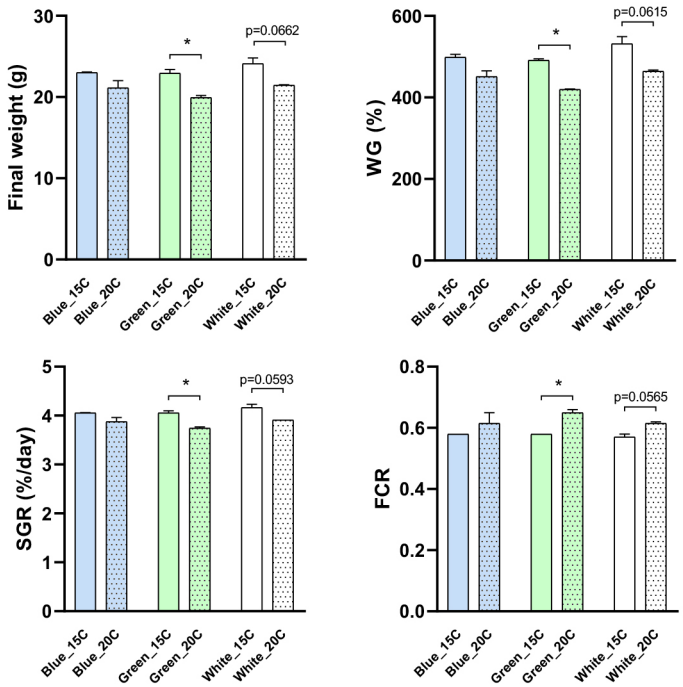


Fig 2. Effect of colour light and temperature on the growth performance parameters. WG (weight gain); SGR (specific growth rate); FCR (feed conversion ratio). *Indicate significant differences between temperatures (P<0.05). Data are shown as mean + standard error of the mean (n=2 tanks).

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# EXPLORING CIDER LEES AS AN ALTERNATIVE INGREDIENT IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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## Introduction

The sustainability of aquaculture is essential for its long-term viability. As such, a significant research area focuses on finding more sustainable alternative ingredients for aquafeeds. The circular economy plays a crucial role, particularly by using by-products as valuable feed inputs. In Spain, cider production reaches approximately 78 million litres annually, with 73% originating from Asturias. This industry generates large quantities of a residual by-product from fermentation and maturation of cider, known as cider lees, which could potentially be used in aquaculture feeds. Cider lees are characterized by their high content of insoluble dietary fibre and different compounds with antioxidant capacity (Rodríguez et al., 2017). The objective of the present study was to evaluate the effect of including cider lees in fish diets, specifically to assess their impact on fish growth and antioxidant capacity.

## Material and methods

To evaluate growth performance, fish were fed diets containing two different levels of cider lees (CL10: 10% and CL20: 20%; Table 1) and a control diet for 88 days in a recirculating aquaculture system. Each diet was tested in triplicate, with 30 fish per tank, and fed to apparent satiety once daily (up to 3%). Following the feeding trial, fish from each dietary group were split into two tanks per diet. One tank was subjected to an acute hypoxia challenge (4 ppm $O_2$/10 min; de Mercado et al., 2018), while the other served as a non-stressed control. Blood samples were collected from six fish per tank at four time points: pre-stress (BASAL), immediately post-stress (0H), and at 2- and 5-hours post-stress (2H; 5H). Samples were collected in heparinized syringes and centrifuged to recover plasma, which was stored at -80°C until analysis. Ferric-Reducing Antioxidant Power (FRAP) was measured in the plasma using the Benzie and Strain (1996) method, with results expressed as μM $Fe^{2}$□. Data were analyzed using a one-way ANOVA followed by Tukey's post hoc test, with a significance level of $P < 0.05$.

**Table 1. Composition of experimental diets**

| *% Ingredients (on dry weight)* | CONTROL | CL10 | CL20 |
|---|---|---|---|
| Fishmeal | 20.0 | 20 | 20 |
| Cider lees | 0.0 | 10 | 20 |
| Soy protein concentrate | 20.0 | 20 | 20 |
| Wheat gluten | 12.0 | 11.5 | 11.5 |
| Corn gluten | 6.0 | 6 | 6.5 |
| Chicken meal | 5.0 | 4.5 | 3 |
| Wheat meal | 18.0 | 9 | 0 |
| Fish oil | 11.0 | 11 | 11 |
| Fish soy | 5.2 | 5.2 | 5.2 |
| Vit & min premix | 1.0 | 1 | 1 |
| Monocalcium phosphate | 0.8 | 0.8 | 0.8 |
| Lysine | 0.3 | 0.3 | 0.3 |
| Threonine | 0.1 | 0.1 | 0.1 |
| Methionine | 0.6 | 0.6 | 0.6 |
| *% Proximal composition (on dry weight)* | | | |
| Protein | 45.6 | 45.1 | 45.5 |
| Fat | 20.2 | 20.9 | 21.4 |
| N-Free extract (NFE) | 26.7 | 26.2 | 25.2 |

## Results and discussion

At the end of the feeding trial, fish reached a final weight of 73.3 ± 2.6 g. No significant differences were found in final weight, specific growth rate (SGR), or feed conversion ratio (FCR) among the dietary groups. After the hypoxia challenge, all fish showed an increase in antioxidant capacity (FRAP) at time 0H, followed by a gradual decrease at 2H and 5H post-stress. However, this decrease was only statistically significant in the control group, suggesting a depletion of the antioxidant system after 5H post-stress. In contrast, fish fed the 20% cider lees diet (CL20) maintained higher FRAP levels throughout the recovery period, showing a significantly higher antioxidant capacity than the other groups at 5 hours post-stress (Fig 1). Non-stressed fish maintained stable FRAP levels over time, with higher values observed in fish fed with cider lees (data not shown). The preliminary results of this study show that including up to 20% cider lees in rainbow trout diets has no negative effects on growth performance and enhances the fish's antioxidant capacity, suggesting its potential as a functional ingredient. Other studies have explored the use of similar by-products, such as grape pomace (solid residues after juice; Peña et al., 2020) and wine lees (Martínez-Antequera et al., 2023) in aquaculture diets, without negative effects on growth and providing functional properties. To our knowledge, this is the first study to investigate the use of cider lees as a sustainable and alternative ingredient in aquaculture feed. While further analysis is needed to understand the effects on various physiological parameters, these preliminary results highlight the potential of cider lees as a functional and alternative ingredient for rainbow trout diets.

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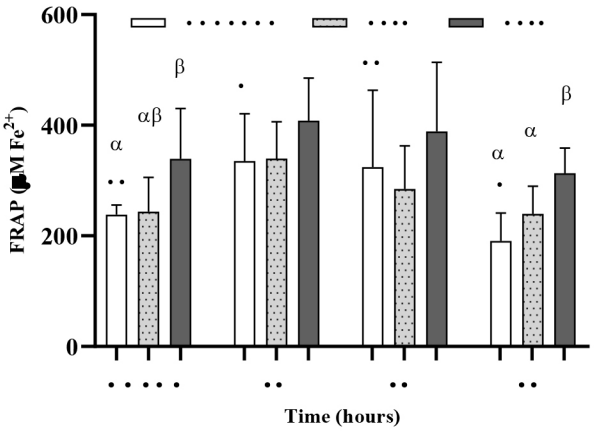


Fig 1. Evaluation of the total antioxidant capacity by FRAP in the plasma blood of rainbow trout (*Oncorhynchus mykiss*) at different time points: pre-stress (basal), 0H (immediately post-stress) and 2H and 5H post-stress. Data are presented as mean ± standard deviation. [a, b] Indicates statistically significant differences ($p < 0.05$) between times within a same diet. [α, β] Indicates statistically significant differences ($p < 0.05$) between diets within a same time.

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# LIVE FROM THE CAGE: GILTHEAD SEABREAM (*Sparus aurata*) RESPONSE TO OPERATIONAL AND ENVIRONMENTAL FACTORS IN A COMMERCIAL SEA FARM

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In order to achieve sustainable and ethical aquaculture practices, it is imperative to gain a comprehensive understanding of the interactions between fish species and their environment. The aquaculture industry is in need of instruments capable of objectively and accurately monitoring fish health and welfare in real time, without disturbing the fish or interfering with standard management practices. Precision livestock farming is a subject that has gathered increased attention given its potential to enhance animal welfare, whilst concomitantly increase production and environmental sustainability (Føre et al. 2018; Brijs et al. 2021). The utilisation of biological sensors, capable of measuring diverse parameters (e.g. depth, acceleration, muscle activity, heart rate), has emerged as a pertinent tool to assess fish health and welfare in response to aquaculture environments and practices (e.g. Carbonara et al. 2019, 2021; Morgenroth et al. 2024; Toomey et al. 2025). The present study evaluated the effectiveness of wireless environmental and accelerometer/pressure sensors in monitoring fish welfare and in gathering insights into relationships between fish acceleration/depth and environmental/farm operational factors.

An experimental trial was conducted within a sea cage of industrial scale at the Rehomare S.r.l. farm in Torre Suda, Italy. In this trial, a variety of environmental sensors were deployed in the water to monitor environmental parameters, namely temperature, oxygen saturation, salinity, tilt (i.e. used as a proxy for water current), and turbidity. Furthermore, we implanted accelerometer or accelerometer/pressure sensors, with a tailbeat mode algorithm, in gilthead sea breams (*Sparus aurata*), thus enabling the measurement of fish acceleration ($m.s^{-2}$), which serves as a proxy for energy expenditure (Alfonso et al. 2021), and depth (m). The experiment was conducted over a period of 305 days, commencing in September 2023 and concluding in September 2024, the experiment being subjected to intermittent interruptions due to technical issues. A total of 40 fish were tagged at different periods during the experiment. All sensors provided real-time data visibility to the farmers, and a total of 1,855,363 detections (acceleration + environmental variables) were obtained after the data was cleaned (i.e. false detections were excluded). A comprehensive data set was compiled, encompassing the quantity of feed administered daily, in addition to any occurrence of disruptions (e.g., feeding, sensor maintenance, or the introduction of tagged fish into the sea cage). Linear mixed models were employed to assess the variation of acceleration according to the period (night/day), the different environmental variables (i.e. temperature, salinity, oxygen, sensor tilt, turbidity), as well as the amount of feed provided and the occurrence of disturbances. The effect of these factors on the switch between aerobic and anaerobic metabolism was also investigated using a binomial generalised linear model.

The utilisation of wireless technology allowed for the acquisition of precise temporal data on various pivotal environmental parameters to explore relationships with fish behaviour/physiology. A range of significant variations were observed across time for the environmental data. For instance, the mean temperature was found to be 27.7 ± 5.7°C, with a range between 13.6 and 29.7°C. Similarly, the levels of dissolved oxygen varied from 48.3 to 126.8%. Overall, fish exhibited clear differences in day/night patterns both in terms of acceleration and depth, with fish being in shallower waters and displaying lower acceleration values during the night. Furthermore, the observed pattern of acceleration across hours indicates a possible anticipatory behaviour in relation to feeding. The study also identified the influence of environmental parameters on fish acceleration. In addition to photoperiod, a significant positive correlation was observed between acceleration and temperature, tilt, turbidity, and dissolved oxygen. Conversely, an inverse correlation was observed between acceleration and salinity or feed supply. In the switch between aerobic and anaerobic metabolism, temperature and disturbances appeared to be particularly important factors. In terms of depth, a positive correlation was observed with turbidity and salinity, and fish were in deeper waters in the absence of disturbance. Conversely, a negative relationship was evident with temperature and oxygen. The results of this study are overall in agreement with those previously obtained for Gilthead seabream in other contexts (e.g. Alfonso et al., 2024; Muñoz et al., 2020; Toomey et al., 2025). However, we provide here various new insights on relationships with new environmental and farm operational parameters and over a longer time period. A more in-depth discussion of these findings is required, with particular emphasis on the season and the inherent variability among individual fish.

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This study provided preliminary insights into the complex interplay between environmental factors and Gilthead seabream acceleration and depth, which are useful information for future welfare prediction indicators. The next steps are the integration of growth data to facilitate the creation of predictive models. The integration of acoustic telemetry technology with environmental sensors provides fish farmers with real-time data, which could function as an alert system for adverse events, enabling timely adjustments to be made to management practices, thus enhancing productivity and fish welfare.

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# GONADAL CYCLES OF THE BIVALVE MUSSELS *Mytilus chilensis* AND *Mytilus galloprovincialis* AT TUMBES COVE, CENTRAL CHILE

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## Introduction

Marine mussels represent an important component of the intertidal and subtidal communities and estuaries, and also of considerable economic importance to aquaculture in many regions of the world (FAO, 2024). The issue regarding the recently published evidence of the presence and potential dissemination of alien mussel species in the Chilean coast, that could cause an impact on the coastal benthic biodiversity and a tremendous damage to the Chilean indigenous mussel (*M. chilensis*) aquaculture growing industry; is a potential problem. Among some of the main features of these *Mytilus* species is the well-known capability to naturally hybridize among them in locations where their distributions overlap (Comesaña et al., 1999; Gardner et al., 2021). Furthermore, a low percentage of "natural hybrids" between these mussels' species have been also described in the literature along the Chilean coast, Larraín et al. (2012), described, a few hybrids between *M. galloprovincialis* & *M. chilensis.* Jilberto et al. (2023) also described at the Strait of Magellan, some hybrids between *M. chilensis* & *M. platensis*. The study of the reproductive cycles of *M. chilensis* and *M. galloprovincialis* at Tumbes Cove where they are found sympatrically is important, to establish the amount of reproductive output of each mussel species and possible synchronous spawning which leads to potential formation of natural hybrids.

## Material and Methods

The recollection of mussels (*M. chilensis* and *M. galloprovincialis*) from the natural beds at Tumbes Cove (Fig 1), where then suspended from a long line in pearls nets 4 months before the experiment. During 17 months 30 mussels from each species were sampled. The samples were transported at 4°C to the laboratory and within 1 day following each field sampling, 20 mussels (38–42 mm s.l.) from each species, were dissected carefully to separate the mantle (and in some the mesosoma) from other soft tissues. The histological procedures were made following Comesaña et al., (1999) (fig. 2). A piece of mantle border (20–30 mg) from each individual was stored (95% ethanol at –20°C) for identification of the species using the diagnostic genetic markers (Illesca et al. 2018). Histological analysis and terminology for the stages of gametogenesis followed Oyarzún et al., (2018).

## Results

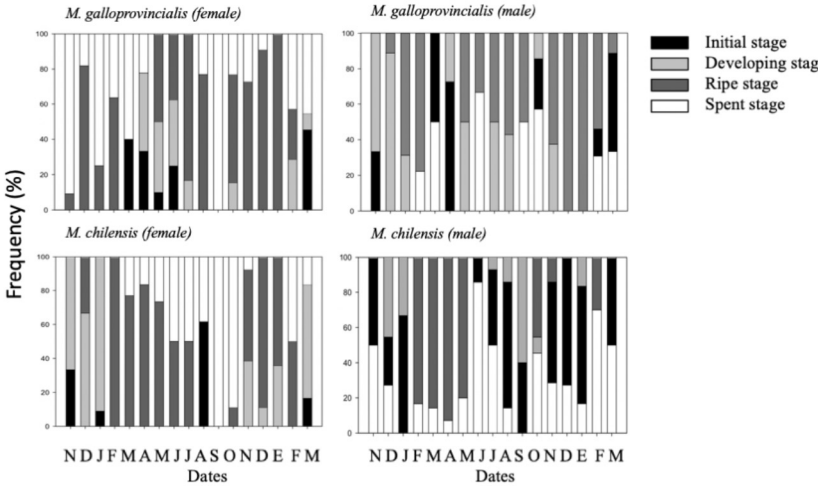


**Fig. 3**: *Mytilus* spp. Frequency distribution of gonad maturation stages in female and male *M. galloprovincialis* and *M. chilensis*.

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Fig. 1. Location sampled, Tumbes Cove.
Upper valves: *M. galloprovinciales*
Lower valves: *M. chilensis*

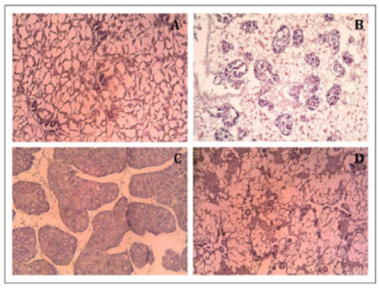


Fig 2. gonad maduration stages
A: Initial. B. Developing
C: Ripe. D: Spend

**Discussion and Conclusions:**

According to the results we observed a high synchronous spawning during winter (July) from both species (Fig. 3). The beginning of the gametogenic cycle for *M. galloprovincialis* was observed in November, just after the spawning (spring) reaching over 40% of individuals (male and female) at the Development stage. Then, towards winter (June) the stages moved from Development to Ripe Stages (Fig 3), having a spawning during July. During august-september, presented another short gametogenesis cycle with Development and Ripe stages following a Spend (spawning) stage during october-november. *M. chilensis*, showed two spawning periods, February-march (summer), and July (winter). The gametogenesis cycle of this species is over a larger period presenting individuals with at Ripe stage for a long period (Fig. 3). The gamete volume fraction (GVF) analysis showed a high prevalence of Ripe Stage gametes during several months sampled, while the a drastic reduction of these, shows spawning periods These results are in accordance to the literature Tarifeño et al. (2006).

**Acknowledgements:** Founded: Research Grant Project FONDECYT 1230212

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# TURBULENCE AND SURFACE WAKES IN A COASTAL AQUACULTURE AREA: INSIGHTS FROM THE SIRENA PROJECT

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## Introduction

The SIRENA project aims to develop innovative monitoring tools for aquaculture farms in Gran Canaria, focusing on external and internal environmental processes. This study compares two oceanographic scenarios—one with an island wake and one without—using a multidisciplinary approach combining satellite imagery, high-resolution model data, and in situ turbulence measurements.

## Methods

Four seasonal oceanographic campaigns were conducted: March 5, April 7, June 30 and July 28; employing a free-falling turbulence profiler (VMP-250) and a multiparameter EXO2 probe to collect vertical profiles of physical and biogeochemical variables. Surface fields and subsurface profiles were obtained from the Puertos del Estado operational ocean forecast model (≈250 m resolution). Sentinel-2 imagery was used to detect and delineate island wake structures (Figure 1).

## Results

Vertical profiles of conservative temperature from in situ measurements and Puertos del Estado model outputs at Station 5 showed discrepancies across seasons (Figure 2). The model generally overestimated temperatures by about 0.3°C in March and surface temperatures in April but underestimated temperatures below 10 meters. On June 30, observed temperatures were up to 2°C higher than the model in upper layers, likely due to inaccuracies in the model's delineation of the island wake boundary. A similar, though less pronounced, pattern was seen on July 28, outside the wake area. Observations showed a warming trend toward summer with stronger stratification later in the year.

Profiles of potential density and turbulent dissipation rate (ε) indicated stronger stratification and higher turbulence below 20 meters on June 30 (with wake) compared to July 28 (without wake). Satellite imagery and model outputs differed notably in the spatial extent of the island wake, affecting sea surface temperature predictions. However, in situ turbulence data were consistent with the wake location observed in Sentinel-2 imagery, supporting the satellite's spatial positioning of the wake.

## Conclusions

The Puertos del Estado model shows limitations in accurately resolving island wake features, impacting environmental assessments. These findings underscore the necessity for higher-resolution models, such as the one developed in the SIRENA project, to improve monitoring and management of aquaculture farms in complex coastal environments.

## Acknowledgements

This project is developed with the collaboration of the Biodiversity Foundation of the Ministry for Ecological Transition and the Demographic Challenge, through the Pleamar Programme, and is co-financed by the European Union through the European Maritime, Fisheries and Aquaculture Fund.

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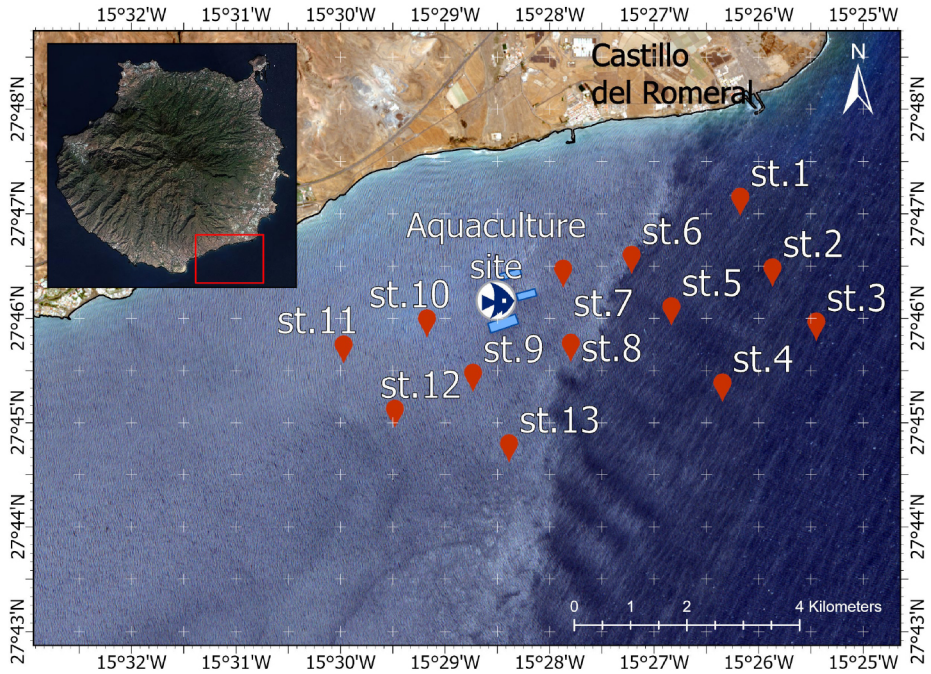


Figure 1. Sentinel-2 image showing surface features associated with the island wake south of Gran Canaria on July 28. Sampling stations and aquaculture sites are marked for reference.

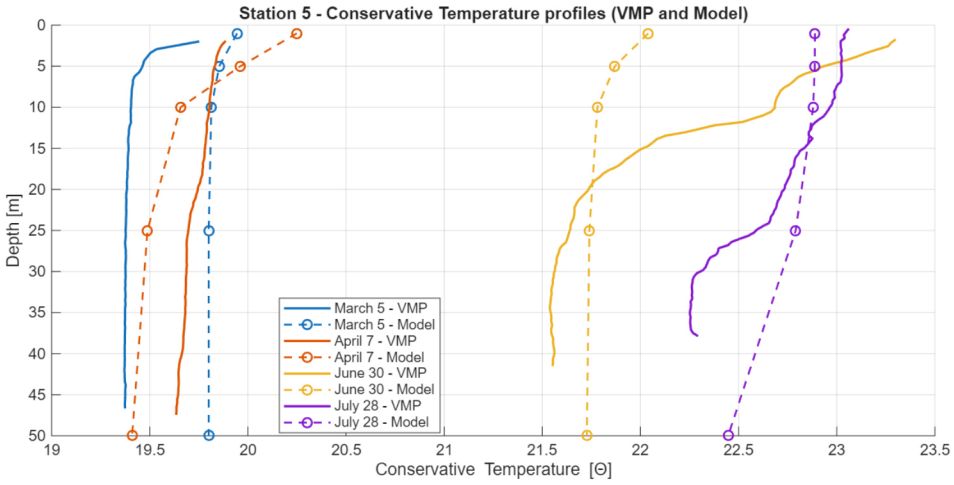


Figure 2. Comparison of in situ conservative temperature profiles (solid lines) and model daily outputs (dashed lines with markers) at Station 5. Colors indicate dates: March 5 (blue), April 7 (orange), June 30 (yellow), and July 28 (purple).

# EFFECT OF THE HIGH TEMPERATURE ON GROWTH AND METABOLISM OF CLAM *Ruditapes decussatus* CULTURED WITH AND WITHOUT SUBSTRATE

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## Introduction

The diversification of cultured species represents a promising strategy for enhancing the economic viability of the aquaculture industry. Within the European Union, this approach has emphasized the importance of some species, such as the grooved carpet clam (*Ruditapes decussatus*), due to its high potential from biological, economic, and gastronomic perspectives (FAO, 2024). Furthermore, sustainable clam aquaculture may contribute to the conservation of European natural populations of this mollusc species, which are currently in decline due to overexploitation, disease, and competition with other bivalve species, such as the Manila clam. These challenges may be exacerbated under global warming scenarios, making it critical to assess the effects of elevated temperatures on the culture of marine invertebrates, whose growth, metabolism, and overall welfare are closely linked to environmental thermal conditions (Rato et al., 2022). In this line, the objective of this study was to evaluate the impact of high environmental temperatures (28-30 °C) on the physiology and metabolism of *R. decussatus*, previously cultured under two conditions: with substrate and without substrate during all life cycle.

## Materials and Methods

*Ruditapes decussatus* specimens were obtained from thermal shock-induced spawning at the IFAPA-Agua del Pino facilities (Cartaya, Huelva, Spain). Juvenile clams were previously reared for 15 months under two distinct conditions: (i) with substrate and (ii) without substrate. Following this initial rearing period, a total of 240 clams were individually measured, weighed, and randomly allocated into 12 perforated rectangular baskets (20 clams per basket), maintaining the respective substrate conditions: 120 clams in six baskets with substrate and 120 clams in six baskets without substrate. These were assigned to four experimental groups (in triplicate): with and without substrate, each maintained at either control temperature (18 °C) or elevated temperature (28 °C) for 28 days. Clams were fed a mixed microalgae diet consisting of *Skeletonema costatum*, *Chaetoceros gracilis*, *Isochrysis galbana*, and *Tetraselmis suecica*. At the end of the thermal exposure period, biometric data were collected, and soft tissue samples from each experimental tank (n =12) were harvested and stored at -80 °C for subsequent biochemical analyses.

## Results and Discussion

Prior to the thermal challenge, clams reared with substrate exhibited superior growth performance and body condition compared to those cultured without substrate. After 28 days of exposure to elevated temperature, significant differences in growth parameters were observed among the experimental groups, with temperature emerging as a key factor. Under both rearing conditions (with and without substrate), high temperature negatively affected soft tissue weight (Table 1). In addition, individuals maintained at 28 °C (High) displayed reduced shell length compared to those held at 18 °C (Ctrl). This detrimental temperature effect was also reflected in the condition index, which reached its highest values in clams maintained at 18 °C, regardless of substrate presence. Concerning intermediary metabolism, no significant differences were detected in triglyceride, cholesterol, or glycogen concentrations among groups. However, glucose levels peaked in clams reared without substrate at the control temperature (Fig. 1A), suggesting increased carbohydrate mobilization to meet energy demands associated with growth, as supported by the elevated condition index in this group. Lactate accumulation was observed in clams exposed to high temperature, irrespective of substrate availability (Fig. 1B). Interestingly, under control temperature, clams reared without substrate also exhibited higher lactate levels than those with substrate. Although lactate is not the primary end-product of anaerobic glycolysis in bivalves (de Zwaan & Eertman, 1996), its accumulation is often associated with muscle fatigue and physiological stress, potentially induced by thermal stress and/or the absence of substrate. Total lipid content was significantly influenced by temperature, with the lowest levels observed in clams exposed to high-temperature conditions (Fig. 1C). This reduction likely reflects increased utilization of lipid reserves to meet the elevated energetic demands required to maintain homeostasis under thermal stress.

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**Table 1.** Growth parameters of *R. decussatus* cultured with and without substrate subjected to a thermal challenge during the 28 days.

| | Substrate | | | No Substrate | | |
|---|---|---|---|---|---|---|
| | **$T_0$** | **Ctrl** | **High** | **$T_0$** | **Ctrl** | **High** |
| **T. W. (g)** | 8.62 ± 1.82$^a$ | 9.21 ± 1.65$^b$ | 8.63 ± 1.54$^a$ | 4.48 ± 1.37$^a$ | 5.81 ± 1.77$^b$ | 4.59 ± 1.26$^a$ |
| **M.W. (g)** | 2.83 ± 0,63$^a$ | 3.20 ± 0.64$^b$ | 2.54 ± 0.53$^a$ | 1.49 ± 0.55$^a$ | 2.04 ± 0.61$^b$ | 1.36 ± 0.44$^a$ |
| **S. W. (g)** | 4.72 ± 0.85$^b$ | 4.43 ± 0.81$^{ab}$ | 4.16 ± 0.67$^a$ | 2.55 ± 0.75$^{ab}$ | 2.83 ± 0.87$^b$ | 2.33 ± 0.68$^a$ |
| **S. L. (mm)** | 34.47 ± 2.41$^a$ | 35.59 ± 2.11$^b$ | 34.76 ± 2.02$^a$ | 27.39 ± 2.94$^a$ | 29.58 ± 2.78$^b$ | 27.92 ± 2.87$^a$ |
| **C. I. (D.W.)** | 59.83 ± 5.23$^a$ | 72.10 ± 7.20$^b$ | 60.27 ± 7.41$^a$ | 53.97 ± 5.28$^a$ | 72.49 ± 7.15$^b$ | 57.67 ± 6.35$^a$ |

Data of growth parameters are shown as the mean ± SD (n=60). Different letters (a, b) in each row denote significant differences (one way-ANOVA and Tukey test, $P \leq 0.05$) among clams reared in $T_0$: Initial time; Ctrl: 18 °C; High: 28 °C for the same substrate condition (with or without). T.W.: Total weight; M.W.: Meat weight; S.W.: Shell Weight; S. L.: Shell length; C. I.: Condition Index = M.W./S.W*100; D.W: Dry weight.

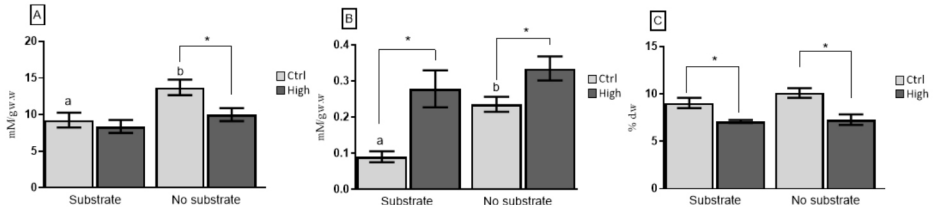


**Figure 1**. Glucose (A), lactate (B), and total lipids (C) values in whole meat of *R. decussatus* cultured with and without substrate, and submitted to different water temperatures (Ctrl: 18 °C; High: 28 °C) for 28 days. Asterisk (*) over the box denotes significant differences (t-Student, $P \leq 0.05$) between clams reared with the same substrate condition (with/without) at 18 °C or 28 °C. Different letters (a, b) over the box denote significant differences among clams reared with the same $T^a$, with or without substrate.

In summary, the results demonstrate that elevated environmental temperatures exert a detrimental impact on growth performance in *R. decussatus*, regardless of substrate availability. While further research is warranted, these findings provide valuable insights into the potential effects of climate change on clam aquaculture productivity.

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# OPTIMIZATION OF *Ruditapes decussatus* CULTURE: EFFECT OF THE SUBSTRATE AND STOCKING DENSITY ON GROWTH AND METABOLISM

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## Introduction

Since the mid-1920s, when intensive harvesting of the grooved carpet shell (*Ruditapes decussatus*) began, its natural populations, despite having a high recovery capacity, have experienced a sharp decline. This has led the main producing countries, i.e., Spain, France, and Portugal, to focus on sustainable aquaculture as a key strategy to improve the economic sustainability of the sector, thereby reducing environmental extractive pressure (FAO, 2024). An optimized farming method is key to the culture's profitability. Thus, determining the optimum stocking density, maintaining a well-organized farming system, and ensuring the availability and quality of substrate are specific technical solutions that facilitate easier intervention in the event of any problem (e.g., pollution, parasitism, abrupt environmental changes, etc.). This study aimed to assess the effects of substrate and stocking density on the physiology and metabolism of *R. decussatus* throughout the entire culture cycle.

## Materials and Methods

*R. decussatus* specimens were obtained from thermal shock-induced spawning at the IFAPA-Agua del Pino facilities (Cartaya, Huelva, Spain). Clams were reared for 15 months in baskets with mesh sizes adapted to each developmental stage, under different culture conditions: (i) with substrate, and (ii) without substrate. Throughout the entire culture cycle, three stocking densities were maintained: Low Stocking Density (LSD; 1000 specimens/m²), Medium Stocking Density (MSD; 2000 specimens/m²), and High Stocking Density (HSD; 3000 specimens/m²). Clams were fed a mixed microalgal diet composed mainly of *Skeletonema spp*. and *Chaetoceros spp.* Rearing conditions, including flow rate, temperature, and oxygen saturation, were monitored weekly. Growth parameters were assessed monthly over the culture period. At the end of the experimental period, final biometric measurements were recorded, and soft tissue samples from each experimental tank (n = 18) were collected and stored at -80 °C for subsequent biochemical analyses.

## Results and Discussion

Clams reared with substrate exhibited enhanced growth performance and improved body condition compared to those cultured without substrate. This difference may be attributed to more favorable feeding conditions, as the presence of substrate facilitates proper shell opening for optimal microalgae filtration and ingestion (Zhang et al., 2023). When substrate is available, clams can rest their valves against the surrounding sediment, allowing the adductor muscles to relax during filtration. Conversely, clams cultured without substrate must maintain continuous adductor muscle contraction to ensure valve alignment, which may reduce filtration efficiency. Regarding stocking density, significant differences in growth parameters were observed among the experimental groups, with high density exerting a negative effect (Table 1). This effect was more pronounced in clams reared with substrate, which exhibited higher growth rates at low stocking density (Table 1).

Regarding metabolic response, the influence of stocking density was more pronounced in clams reared with substrate. Under both culture conditions (with and without substrate), no significant differences were observed in glycogen and total lipid levels. However, cholesterol, triglycerides, and glucose (also without substrate) levels decreased in clams reared with substrate at high density (HSD). Additionally, lactate concentrations reached their peak in these clams (Table 2). This metabolic profile may reflect increased spatial competition, which likely impairs feeding efficiency and consequently limits growth. The accumulation of lactate can thus be associated with physiological stress, potentially induced by elevated competitive pressure.

These findings, which have direct implications for aquaculture practices, highlight the importance of optimizing rearing conditions for *R. decussatus*. Based on the present results, a maximum recommended stocking density of ≤ 2000 individuals/m² on substrate is proposed to enhance the profitability and sustainability of this species' cultivation.

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**Table 1.** Growth parameters of *R. decussatus* cultured with and without substrate and maintained at several stocking densities during the 15 months. Data on growth parameters are presented as the mean ± SD (n = 24). Different letters in each row denote significant differences (one way-ANOVA and Tukey test, $P \leq 0.05$) among clams reared at Low (LSD), Medium (MSD), and High (HSD) Stocking Density for the same substrate condition (with or without) for a 15-month period

| | Substrate | | | No Substrate | | |
|---|---|---|---|---|---|---|
| | **LSD** | **MSD** | **HSD** | **LSD** | **MSD** | **HSD** |
| **T. W. (g)** | 8.63 ± 1.88$^{c}$ | 7.02 ± 1.80$^{b}$ | 5.84 ± 1.82$^{a}$ | 4.50 ± 1.41$^{b}$ | 4.25 ± 1.25$^{b}$ | 3.86 ± 1.16$^{a}$ |
| **M.W. (g)** | 2.83 ± 0.64$^{b}$ | 2.29 ± 0.81$^{a}$ | 2.04 ± 0.65$^{a}$ | 1.49 ± 0.55 | 1.22 ± 0.81 | 1.23 ± 0.38 |
| **S. W. (g)** | 4.72 ± 0.85$^{b}$ | 4.02 ± 1.17$^{a}$ | 3.47 ± 0.92$^{a}$ | 2.55 ± 0.75 | 2.24 ± 0.69 | 2.20 ± 0.45 |
| **S. L. (mm)** | 34.70 ± 2.55$^{c}$ | 32.27 ± 2.96$^{b}$ | 30.07 ± 3.27$^{a}$ | 27.39 ± 2.94$^{b}$ | 26.96 ± 2.88$^{b}$ | 26. 20 ± 2.50$^{a}$ |
| **C. I. (D.W.)** | 59.83 ± 5.23 | 56.13 ± 6.62 | 58.11 ± 7.41 | 53.97 ± 5.28 | 57.62 ± 6.83 | 55.27 ± 6.68 |

T.W.: Total weight; M.W.: Meat weight; S.W.: Shell Weight; S.H.: Shell Length; C.I.: Condition Index = M.W./S.W*100; D.W.: Dry weight.

**Table 2.** Biochemical parameters of *R. decussatus* cultured with and without substrate and maintained at several stocking densities during the 15 months. Data are shown as the mean ± SD (n = 12). Different letters in each row denote significant differences (one way-ANOVA and Tukey test, $P \leq 0.05$) among clams reared at Low (LSD), Medium (MSD), and High (HSD) Stocking Density for the same substrate condition (with or without) for a 15-month period.

| | Substrate | | | No Substrate | | |
|---|---|---|---|---|---|---|
| | **LSD** | **MSD** | **HSD** | **LSD** | **MSD** | **HSD** |
| **Cholesterol** | 3.54 ± 0.66$^{a}$ | 4.59 ± 1.15$^{b}$ | 3.09 ± 0.86$^{a}$ | 4.67 ± 1.47 | 4.42 ± 1.00 | 5.22 ± 1.75 |
| **Triglycerides** | 1.45 ± 0.50$^{ab}$ | 1.87 ± 0.63$^{b}$ | 1.15 ± 0.46$^{a}$ | 2.72 ± 1.31 | 2.61 ± 1.22 | 3.05 ± 1.57 |
| **Glucose** | 3. 30 ± 0.56$^{a}$ | 3.43 ± 0.49$^{a}$ | 4.39 ± 0.24$^{b}$ | 3.93 ± 0.71$^{b}$ | 3.81 ± 0.45$^{b}$ | 2.81 ± 0.36$^{a}$ |
| **Lactate** | 0.35 ± 0.17$^{a}$ | 0.46 ± 0.15$^{ab}$ | 0.52 ± 0.17$^{b}$ | 0.58 ± 0.29 | 0.54 ± 0.15 | 0.62 ± 0.32 |
| **Glycogen** | 12.07 ± 1.11 | 11.72 ± 0.88 | 9.93 ± 1.13 | 13.70 ± 1.78 | 11.58 ± 1.57 | 13.11 ± 1.04 |
| **Total lipids** | 6.91 ± 0.66 | 6.90 ± 0.38 | 7.53 ± 0.59 | 7. 18 ± 0.78 | 6.71 ± 0.62 | 6.91 ± 0.44 |

Cholesterol, triglycerides, glucose, lactate, and glycogen are shown as mmol $g^{-1}$ w.w. Total lipids (%): percentage of lipids concerning the total dry weight of the sample analysed.

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# THE AQUALEX MULTIMEDIA CONSORTIUM AND THE CONTRIBUTION OF MARGARET ELEFTHERIOU

E. Mente[1*], Eilleen Dillane[2] and Lluis Tort[3]

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**AQUALEX Multimedia Consortium Ltd. (AMC)** was founded in 1996 in Dublin, Ireland, as a not-for-profit organization aimed at promoting the outcomes of the European Commission **LINGUA** Programme project, **AQUALEX**. The organization is managed by a Board of Directors elected from the original AQUALEX LINGUA project partners and subsequent collaborators. AMC's mission is to provide high-quality multilingual aquatic science resources that facilitate communication within Europe's diverse scientific community, with particular focus on supporting scientists in training.

Since its inception, AMC has made significant contributions to European science, particularly in the development of interactive learning tools for the aquatic sciences. Notably, AMC led to the publication of the **AQUALEX** Glossary in 1997 by John Wiley & Sons, New York. In 1999, AMC expanded its multilingual offerings by launching the **AQUALEX Aquaculture Glossary** online, available in seven key languages for the aquaculture sector (English, French, Spanish, Norwegian, Greek, Italian, and German). Further efforts saw the creation of a **Virtual Learning Environment**, featuring an extensive resource repository that includes open-access teaching and learning tools in various languages, such as English, French, Spanish, Norwegian, Greek, Italian, Portuguese, and Swedish. AMC has consistently supported the development of multilingual aquatic science materials, which are used in both e-learning and academic contexts. These materials include language learning tools tailored to the aquatic science sector, but applicable beyond this field as well.

In 2008, in collaboration with **AquaTT**, AMC established an award in honor of Dr. **Lindsay Laird**, recognizing the most innovative poster submitted by a student in aquaculture and aquatic sciences. This award, supported by publishers Wiley/Blackwell, includes a selection of aquaculture publications donated annually to the winner. Additionally, in 2023, the **Richard Fitzgerald Award** was presented at the Environ Conference held in ATU Letterkenny, in association with the **Environmental Science Association of Ireland (ESAI)**. Later that year, at **HCMR Crete**, **Professor Magoulas** presented **Professor Athanasious Eleftheriou** with a plaque commemorating his significant contributions to the institute.

It was with profound sadness that AMC Ltd. learned of the sudden passing of **Margaret Eleftheriou** in October 2024. Margaret, the founding director of AMC, had an extensive educational career in both the UK and Greece, spanning secondary and tertiary levels. From 1979 to 1992, she played a pivotal role in curriculum development in Scotland. From 1987 to 2001, she taught English for Specific Purposes (ESP) in the Biology Department at the University of Crete. Margaret was a key partner in the LINGUA AQUALEX project, and her dedication to developing an accurate, up-to-date, and multilingual aquaculture glossary was instrumental in its early success. As a founder and chairperson of AMC from its establishment in 1996 until her passing, Margaret tirelessly promoted the organization's mission. She was instrumental in launching the **E-Aqualex Aquatic Sciences e-Learning Toolset**, demonstrating her boundless energy, dynamism, and enthusiasm.

Margaret was a cherished colleague, always generous with her time, knowledge, and expertise. She was deeply committed to research and education, contributing to several EU projects related to her scientific interests. Through her work as a special consultant at the **Institute of Marine Biology of Crete**, she contributed to a series of educational videos and CD-ROMs, including the **AQUALEX** aquaculture glossary (now in 8 languages), **Basic Techniques in Fish Haematology**, and **Spotlight on Greek Marine Science** (available in both English and Greek).

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Her involvement in the **SOCRATES Thematic Network AQUATNET** from 1996 to 2015, as well as her contributions to numerous EU educational projects (such as AQUALEX, ONEDIN, ORION, BIODAQUA, and PESCALEX), were critical in expanding the **AQUALEX** glossary into areas like **Fish Diseases** and **Marine Pollution**. She played a key role in the **WAVE** initiative and the **VALLA** project and contributed to the **AQUATT** submission on **ECVET** (European Credit Transfer for Vocational Education and Training). Her work also led to her nomination as the aquaculture representative in the **EU 2020 ESCO (European Skills/Competences Qualifications and Occupations) Initiative**, which she actively participated in until 2016.

Margaret's expertise in **Vocational Education and Training** (VET) Learning Outcomes made her an invaluable contributor to the **MATES project** (2018–2022), which aimed to foster the European Blue Economy through a Marine Technology Skilling Strategy. She also developed four AMC language courses for the **Marine Training** website and participated in the **EU-funded FLORES project** (2023-2024), where she developed a pilot course, **Introduction to Offshore Renewable Energy**, available in both Spanish and English. Her final focus was on **Ocean Literacy**, and she worked on initiatives to support the **Network of European Blue Schools**. Margaret's dedication to the future of our planet and her vision for the advancement of aquatic sciences were evident in her ongoing work with AMC.

The AMC Board of Directors is deeply honoured to have collaborated with Margaret. She embodied friendship, generosity, and dedication to making a difference in the lives of young students and professionals. Margaret's legacy will continue to inspire all who knew her and worked alongside her. Thank you, Margaret, for the privilege of knowing you and for all your remarkable contributions to the aquaculture and aquatic science fields.

# PRELIMINARY GROWTH DATA ON THE SPIDER CRAB *Maja brachydactyla* JUVENILES OBTAINED IN HATCHERY

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## Introduction

The spider crab species, *Maja brachydactyla* (Herbst, 1788), is the most commercially valued in Spain, with Galicia being the producing region par excellence, with catches of 485,604 kg (€6,037,005) during 2024 (pescadegalicia.gal). Overexploitation of the species was detected since 2002, since more than 90% of the market stocks come from fishing (Freire et al., 2002). As it is not a species produced through aquaculture, the culture of this species in hatcheries could alleviate its scarcity on the market and, in turn, serve to repopulate natural populations that are unstructured. The objective of this work is to determine the optimal cultivation protocol for this species in a hatchery, which would be applicable to the other species of spider crab found on the Mediterranean coast of Spain, *Maja squinado*, whose populations are at risk of extinction (UNEP, 1996).

## Materials and methods

Se compró una hembra ovada de *M. brachydactyla* en la lonja de Canido, se transportó hasta la ECIMAT de Toralla en un contenedor con agua de mar y were placed in square 1000 L fibre glass tanks in a flow-through system. Larval culture was done in duplicate, in 1,000 L square tanks. After a volumetric counting, a total of 50,000 zoeas were placed in each tank. Zoeas and megalopas were fed enriched Artemia on a daily basis at concentrations of 1-3 ind/ml. A continuous small flow of microalgae (T-Iso) was used to enrich water in the culture systems. Water temperature varied between 14 and 17°C. Photoperiod was 12:12 light/darkness. At the end of the pelagic phase, larval survival and the total of the benthic juveniles were determined by individually counting all remaining animals in the tanks. Growth of juvenile individuals was determined by measuring the total carapace length (mm), measured from the frontal peak and the telson (n = 20 each) at 17,31,48,54,71,101,118 and 134 DAH. Individual sizes in the two replicates at the end of the measurement period were compared using an ANOVA to determine significant differences ($p < 05$).

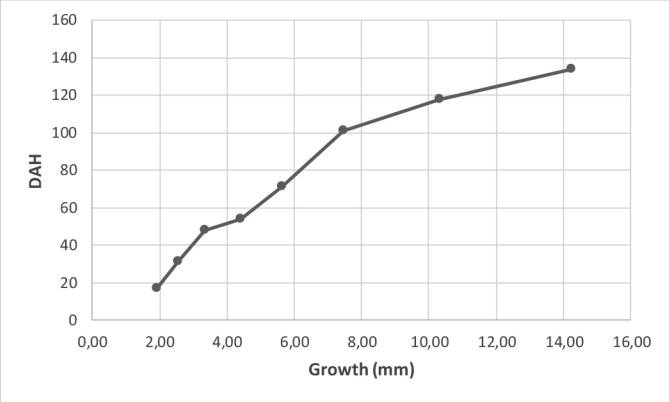


**Figure 1. Growth data of juveniles from 17 DAH**

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An ovate female of *M. brachydactyla* was purchased at the Canido fish market, transported to the ECIMAT in the Toralla Island (Vigo) in a container with seawater and placed in square 1000 L fiber glass tanks in a flow-through system. Larval culture was done in duplicate, in 1,000 L square tanks. After a volumetric counting, a total of 50,000 zoeas were placed in each tank. Zoeas and megalopas were fed enriched Artemia on a daily basis at concentrations of 1-3 ind/ml. A continuous small flow of microalgae (T-Iso) was used to enrich water in the culture systems. Water temperature varied between 14 and 17°C. Photoperiod was 12:12 light/darkness, $O_2$= 7,22 mg/L, salinity= 35,1 pps. At the end of the pelagic phase, larval survival and the total of the benthic juveniles were determined by individually counting all remaining animals in the tanks. Growth of juvenile individuals was determined by measuring the total carapace length (mm), measured from the frontal peak and the telson (n = 20 each) at 17,31,48,54,71,101,118 and 134 DAH. Individual sizes in the two replicates at the end of the measurement period were compared using an ANOVA to determine significant differences ($p < 05$).

**Results**

On average, zoeas I turned to zoea II megalopa in 5 DAH and from zoea II to megalopa in 10 DAH; from megalopa to juveniles lasted 11-13 days. Average survival was 51%, a very high data compared with survival obtained in the bibliography, for example Pazos et al., (2018) obtained an average survival of 13% from megalopa to juvenile. Figure 1 shows the growth data of obtained juveniles from 17 days after hatching.

As you can see in the figure 1, spider crab juveniles obtained in hatchery, reach a size of 14,22 mm at 134 days after hatching.

**Conclusion**

The cultivation of the spider crab *Maja brachydactyla* in hatcheries is a viable alternative for supplying the national market throughout the year, as well as for obtaining juvenile individuals that allow repopulating natural banks that are diminished or unstructured. The cultivation methodology can be extrapolated to the species *Maja squinado*, which is in danger of extinction on the Mediterranean coast of Spain.

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# AQUACULTURE UNDER CLIMATE CHANGE: HOW SKIN MICROBIOME DISRUPTION INFLUENCES WINTER ULCER INFECTION IN ATLANTIC SALMON

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**Introduction**

The aquaculture sector's long-term sustainability is threatened by the effects of climate change, which are not just a future, but a present reality. Changing environmental factors increase the vulnerability of Atlantic salmon to infections such as winter ulcer disease (WUD), which represents a significant challenge in salmon farming, particularly during the sea phase of production. WUD refers to the infection by the bacterium *Moritella viscosa*. However, other species, such as *Aliivibrio wodanis* and *Tenacibaculum spp*., have been isolated from winter ulcer outbreaks. The skin mucosal microbiome constitutes the first barrier of defence against infections by environmental opportunistic pathogens. Research concerning this field has implications for aquaculture sustainability, as shifts in microbial composition (i.e., dysbiosis) are linked to disease outbreaks. Thus, this study aimed to characterize the changes in Atlantic salmon (*Salmo salar*) skin microbiota when subjected to multiple climate change-related environmental stressors and infection by *Moritella viscosa*.

**Materials and Methods**

Smolts were reared under three environmental conditions for 92 days. In the first condition, fish were maintained at 12°C during the whole trial (Control "C" group). The second condition simulated a period of elevated temperatures and was repeated throughout the trial. Here, fish were maintained for five days at 12°C with a gradual increase of temperature during five days up to 17°C, then this temperature was maintained for 10 days, and after this period, it progressively declined to 12°C within 5 days. This temperature fluctuation was repeated three times (Increased temperature (heat) "H" group). In the third condition, temperature changes were maintained as in the H group, with an additional decrease in the percentage of available oxygen to 70% when water temperature reached 17°C (Increased temperature + limited oxygen "HO" group). Subsequently, all fish were transferred to two tanks in a common garden trial, and after 6 days of acclimatization, fish from one of the tanks were infected with *Moritella viscosa* through bath challenge ("I"). Skin mucus samples were collected after 4 and 14 days post-exposure and were analysed through ONT MinION V1-V3 16S rRNA amplicon sequencing and standard analytical pipelines.

**Results**

Bacteroidota was the most abundant phylum among all groups, constituting more than 29.4% of the total bacterial abundance in salmon skin, followed by Pseudomonadota (21.9%), Actinomycetota (18.7%), and Bacillota (6.9%). Interestingly, no significant differences were found in the alpha and beta diversity between the different tested conditions (C, H, HO). However, the genera *Staphylococcus*, *Enhydrobacter,* and *Chryseobacterium* were significantly higher when comparing the H and HO groups with the C group. On the other hand, the bacterial challenge drove significant differences in beta diversity, where several genera, including *Moritella,* significantly changed their abundance between the two sampling points (Fig.1). Interestingly, the abundance of this genus shifted over time, dramatically decreasing in the 14-day sampling, leading to the overgrowth of other taxa such as the genus *Nonlabens* from the family Flavobacteriaceae.

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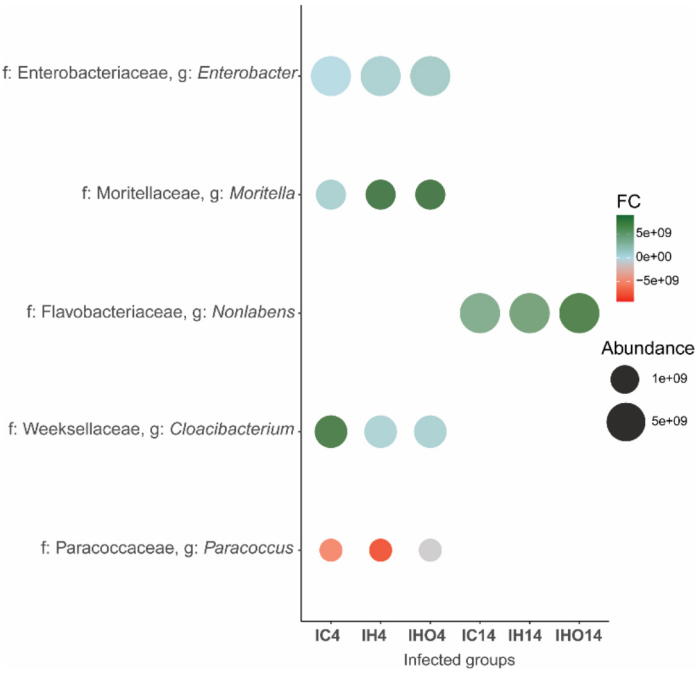


**Fig.1:** Dot plot representing differentially abundant taxa upon *M. viscosa* challenge. Only taxa significantly changing in the three conditions are shown. The colour scale represents the log 2-fold change in abundance relative to the control group. The size of the dots represents normalized counts in each group.

## Conclusions

So far, the experimental infection with *Moritella viscosa* has provided insights into the microbial dynamics associated with WUD through time and while facing different climate change environmental stressors. Increased temperature, in combination with or without decreased oxygen levels, did not dramatically affect skin microbiota or worsen the outcome of the disease. However, these conditions facilitated the increased abundance of opportunistic bacterial taxa like *Staphylococcus* or *Chryseobacterium*. Upon infection, the microbiota in the ulcers changed from colonization by the initial pathogen, *M. viscosa*, to a significant overgrowth of a member of the Flavobacteriaceae family. This effect was probably due to the temperature being too high for *M. viscosa* to prevail, and the ulcer environment favouring the overgrowth of other more adapted genera. A deeper analysis of the microbial composition of the ulcers, their progression with time and temperature, and their functions is needed to determine the roles of key microbial players, with special focus on this microbial shift concerning *Moritella* and the genus *Nonlabens*, which has not been well described as a fish pathogen. Of note, several studies have linked a previously uncharacterized Flavobacteriaceae to infections in fish, indicating that there may be unknown pathogenic genera or species within this family that require further investigation.

## Funding

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# MICROBIOBAL COMPOSITION AND DIVERSITY IN INTESTINE OF SIBERIAN STURGEON (*Acipenser baerii*) FED DIETARY METHANE-UTILIZED BACTERIA (*Methylococcus capsulatus*)

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## Introduction

Methanotroph (*Methylococcus capsulatus*) bacteria protein (MCP) has been proposed as a sustainable and affordable protein source for aquaculture fish species. Studies on Japanese yellowtail (*Seriola quinqueradiata*) (Biswas et al., 2020), white shrimp (*Litopenaeus vannamei)* (Chen et al., 2021), seabass (*Lateolabrax maculatus*) (Yu at ela., 2023), among others, have showed positive effects in terms of growth performance, immunity, and other physiological indices. Siberian sturgeons is an economically important aquaculture fish across global, but the effects of dietary MCP on physiological indcies and gut microbiota have not been tested. This study aimed to to use amplicon sequencing technology to characterrise the gut microbiota of Siberian sturgeons fed on four different diets with increasing MCP inclusion levels, in a recirculating aquaculture system.

## Materials and methods

Four isoproteic (52 %), isolipidic (10%), and isoenergic (18.4 Mj/kg) diets, where MCM replaced fishmeal at 0, 25, 50, and 75% (Abbreviated diets: UNI0; UNI25; UNI50 and UNI75) were formulated and extruded in a 3 mm diameter. Five hundred and forty Siberian sturgeon (23.15±0.35g) were randomly assigned to 300 L tanks (45 fish/tank) and fed four diets (three replicates per diet) for 70 days. The posterior intestine (n = 6 fish and 9 fish/diet, respectively) was dissected and sampled for digesta at the start and end of the feeding trial. Nucleic acid extraction, amplicon library preparation, sequencing, and bioinformatics were performed according to previous publications (Nweze et al., 2024).

## Results

### *Microbial composition*

Fusobacteriota, Firmicutes, and Proteobacteria were the predominant phyla in the intestinal microbiota of Siberian sturgeon, irrespective of dietary treatment (Fig. 1A). The Kruskal–Wallis one-way analysis revealed no significant differences in the relative abundance of phyla among the dietary groups ($P > 0.05$).

At the genus level, *Cetobacterium*, *Clostridium*, *Plesiomonas*, and *Aeromonas* were the most dominant across all experimental groups. Similarly, no significant differences were observed in the relative abundance of genera among the diets ($P > 0.05$). However, a comparison of genus-level abundance between the initial samples and those collected after the 70-day feeding trial revealed a significant shift in bacterial composition, as determined by the Wilcoxon signed-rank test ($P < 0.05$).

### *Alpha diversity*

There were no significant differences in the alpha diversity of the microbial communities in the experimental diets, as measured by the Shannon (Kruskal-Wallis test followed by Dunn's post-hoc test, $P = 0.073$), and Simpson index ($P = 0.064$).

### *Beta diversity*

Principal Coordinate Analysis (PCoA) based on Bray–Curtis dissimilarity was conducted using the relative abundance of ASVs with (Fig. 3A) and without (Fig. 3B) initial intestinal samples. PERMANOVA analysis revealed no significant difference in bacterial community structure among dietary treatments ($P = 0.300$).

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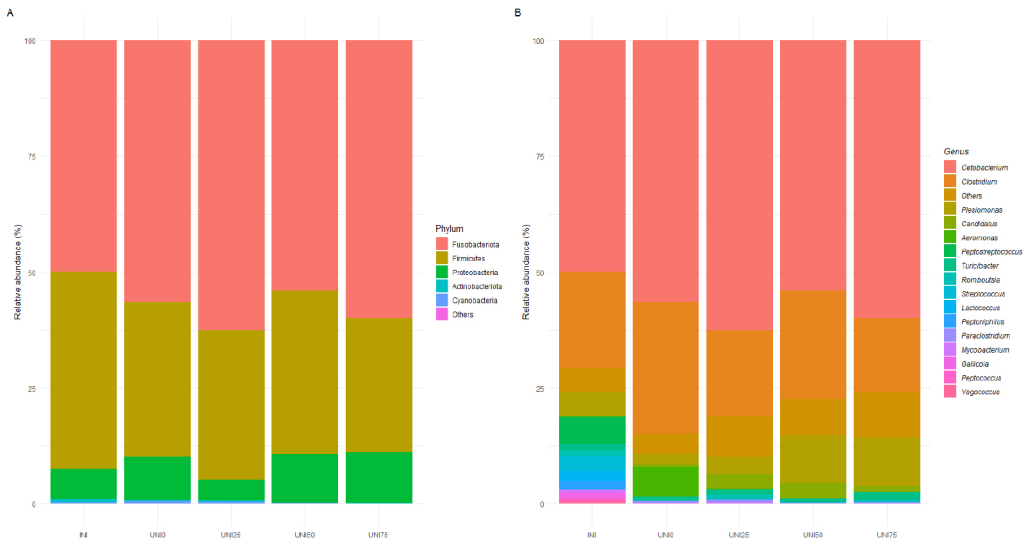


**Fig. 1.** Stacked bar plot showing the bacteria's relative abundance at the phylum (A) and genus (B) level in sturgeon. INI indicates a digesta sample of Siberian sturgeon at the start of the trial (n = 6); CON, UNI25, UNI50, and UNI75 represent experimental diets.

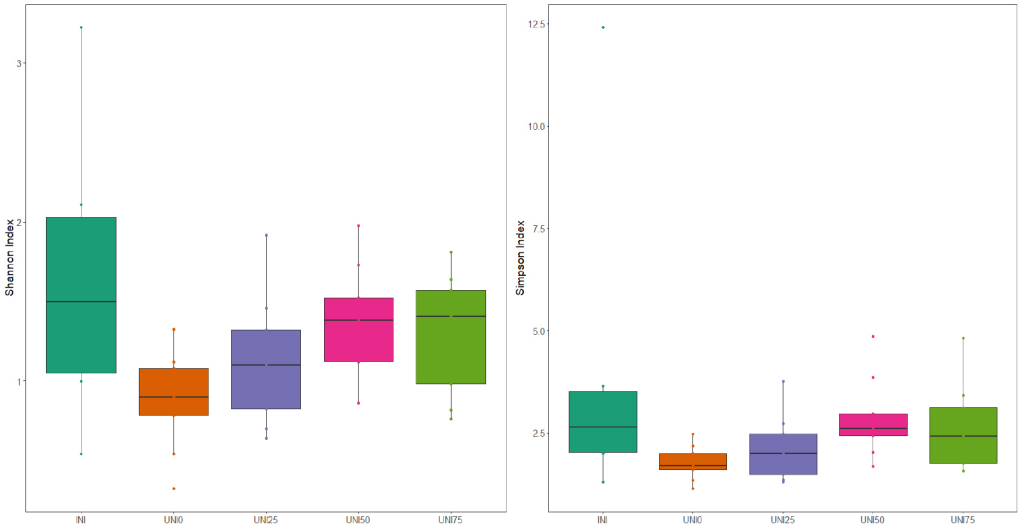


**Fig. 2.** Alpha diversity measured by Shannon and Simpson indexes. Data are shown as box plots (n = 9). The horizontal line indicates the median, and the whiskers indicate the lowest and highest points within the lower or upper quartile interquartile ranges.

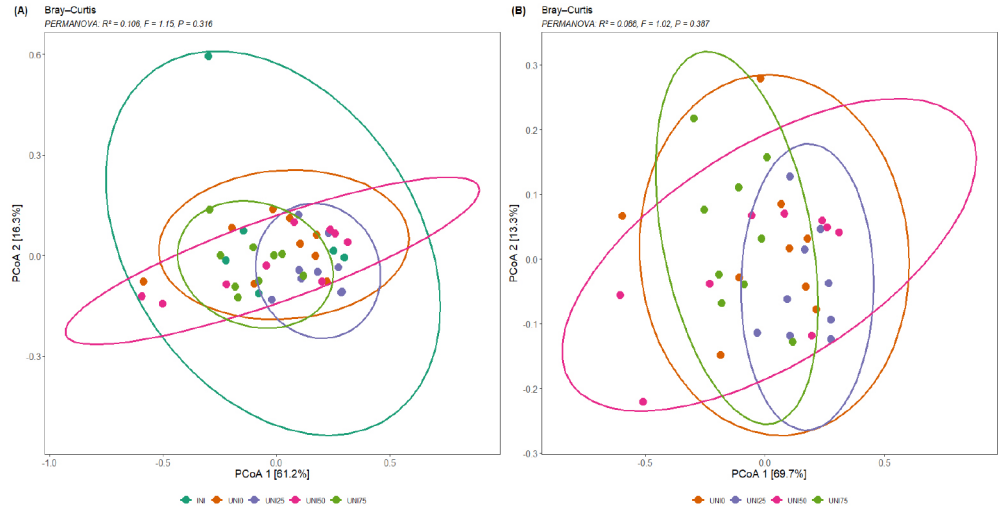


**Fig. 3.** Principal coordinates analysis of intestine microbiota, with (A) and without (B) initial digesta samples (n = 9) based on the Bray-Curtis metric. INI indicates digesta sample of Siberian sturgeon at the start of the trial (n = 6); UNI0, UNI25, UNI50, and UNI75 represent experimental diets.

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Conclusions

- The microbial composition, at phyla and genus levels, in Siberian sturgeon intestine exhibited a significant shift following the 70-day feeding trial, irrespective of diet.
- Dietary MCM had no significant effect on the intestinal bacterial community of Siberian sturgeon.

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# LAND-BASED PILOT IMTA: USING SEABASS HATCHERY EFFLUENT TO GROW SEAWEEDS IN A MEDITERRANEAN AQUAFARM

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## Introduction

Effluent from marine hatcheries, such as those rearing Mediterranean seabass (*Dicentrarchus labrax*), still after treatment. contains considerable levels of inorganic nutrients which could contribute to coastal eutrophication. Integrated multitrophic aquaculture (IMTA) offers a sustainable solution by incorporating extractive species to recover nutrients. The green macroalga *Ulva* spp., with its high nutrient uptake efficiency and rapid growth under variable Mediterranean conditions, is an ideal candidate for such systems (Shpigel et al, 1993).

This work evaluates the cultivation of *Ulva* using seabass hatchery effluent to remove inorganic nutrients while producing biomass for biomaterial applications. We quantify nutrient removal performance, assess biomass yields, and discuss the potential of integrating *Ulva* production into IMTA frameworks to enhance environmental sustainability and circular bioeconomy outcomes in Mediterranean aquaculture.

## Materials & Methods

A land-based pilot IMTA unit was set up at the Kefalonia Fisheries SA finfish hatchery on Cephalonia Island, Greece, to cultivate green seaweed (*Ulva* sp.) using hatchery effluent from sea bass (*Dicentrarchus labrax*) broodstock and juvenile tanks. *Ulva* sp. seedlings sourced locally were loaded at 1 kg/m$^3$ into translucent HDPE tanks (1 m$^2$ each) supplied with sedimentation tank water (the final stage of the hatchery's biological water treatment process), rich in inorganic nutrients, at a 20-minute residence time. Each two-week cultivation cycle included monitoring light, pH, DO, and nutrient levels. All biomass was harvested by net, sun-dried in a greenhouse, weighed, and stored at the end of each cycle.

Light (lux), pH, DO (mg/L) and salinity data were averages of daily measurements (2points, morning-noon). Analyses for nutrients were carried out twice per week. Yield represents the final biomass harvested after two weeks minus the initial loading. Daily growth was calculated from the formula % per day $=100*LN(X_t/X_{t0})/t\text{-}t_0$.

## Results & Discussion

Table 1 shows that cultivating *Ulva* with treated hatchery effluent could be promising if optimised. Biomass output depended on changing ambient factors and nutrient input from the effluent. Nutrient output data were inconsistent, so further experimentation is needed. Generally, dissolved inorganic nitrogen recovery was highest when daily seaweed growth was high (94.43 ± 3 mg/L), medium at moderate growth (65.84 ± 7.08 mg/L), and low at low growth (7.58 ± 1.61 mg/L), though this pattern requires more validation through a different experimental approach (Shpigel personal communication).

Yields and daily growth rates matched the highest values reported in literature (18-20% for land-based *U. lactuca* on fish effluent; Neori et al., 1991; Drawbridge et al., 2024). Optimisation could further improve nutrient recovery and biomass output. Since using animal waste–derived biomass for food or feed is challenging to license, ongoing trials are exploring biomaterial applications such as biofertiliser, biostimulants, and biocomposites, which may be financially viable for the modest output of a typical Mediterranean marine finfish hatchery.

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Table 1. Results of bi-weekly cultivation cycles of *Ulva* sp., on hatchery effluent. Data from one representative tank from 26/2 until 4/8/2025. First row data are averages and second row data are standard deviations

| no | Days | lux | T | S | pH | DO | DIN | PO4 | yield kg/m3 | %/day |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 14 | 16,569 | 16.08 | 31.45 | 7.87 | 7.50 | 0.98 | 0.80 | 1.25 | 5.79 |
| | | 5,462 | 0.95 | 1.07 | 0.20 | 0.36 | 0.12 | 0.85 | | |
| 2 | 14 | 20,628 | 16.83 | 31.93 | 7.96 | 7.37 | 0.63 | 1.40 | 0.73 | 6.40 |
| | | 9,049 | 0.79 | 1.64 | 0.15 | 0.48 | 0.21 | 0.92 | | |
| 3 | 11 | 24,686 | 17.59 | 32.41 | 8.05 | 7.24 | 0.76 | 1.35 | 0.57 | 1.19 |
| | | 12,637 | 0.63 | 2.22 | 0.10 | 0.59 | 0.30 | 2.99 | | |
| 4 | 14 | 26,185 | 19.46 | 26.00 | 8.07 | 6.77 | 1.76 | 2.51 | 2.87 | 5.92 |
| | | 11,471 | 0.89 | - | 0.14 | 0.21 | 0.98 | 3.63 | | |
| 5 | 11 | 32,239 | 20.26 | 26.00 | 8.16 | 7.07 | 1.10 | 0.01 | 13.95 | 21.93 |
| | | 14,521 | 1.48 | - | 0.22 | 0.88 | 0.12 | - | | |
| 6 | 19 | 32,218 | 21.14 | 26.00 | 8.17 | 7.01 | 1.18 | 0.01 | 14.75 | 13.30 |
| | | 17,610 | 0.95 | - | 0.25 | 0.71 | 0.05 | - | | |
| 7 | 13 | 20,619 | 23.35 | 30.78 | 8.21 | 6.90 | 1.20 | 0.83 | 12.34 | 18.35 |
| | | 13,750 | 1.63 | 2.53 | 0.21 | 0.67 | 0.47 | 0.82 | | |
| 8 | 13 | 19,750 | 24.08 | 28.37 | 8.28 | 6.70 | 0.85 | 1.65 | 12.12 | 17.47 |
| | | 13,284 | 1.34 | 0.49 | 0.21 | 0.50 | 0.27 | 1.35 | | |
| 9 | 15 | 19,258 | 25.03 | 28.23 | 8.13 | 6.86 | 2.01 | 0.10 | 0.73 | 3.12 |
| | | 13,128 | 2.18 | 2.10 | 0.23 | 0.83 | 0.42 | - | | |
| 10 | 14 | 23,542 | 24.13 | 26.79 | 8.16 | 6.67 | 1.39 | 1.73 | 3.04 | 8.85 |
| | | 22,893 | 2.00 | 0.77 | 0.17 | 0.46 | 0.69 | 1.96 | | |

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# NUMERICAL ANALYSIS OF NOVEL OFFSHORE AQUACULTURE SYSTEMS

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## Introduction

Offshore structures for finfish aquaculture must meet high standards for reliability and animal welfare. Numerical modelling can help assess alternative designs and find solutions that ensure structural reliability in challenging offshore environments. However, this alone is insufficient. For fish welfare, it is also crucial to maintain adequate space, water quality, and dissolved oxygen (DO) levels within the sea cage. Comprehensive analysis tools are needed to evaluate and optimise new aquaculture systems for their ability to withstand offshore environmental loads while promoting fish welfare. Currently, such tools are absent in both offshore engineering and traditional coastal aquaculture technology.

To address this gap, SINTEF Ocean has developed numerical techniques for modelling and integrated analysis of flexible aquaculture structures and mooring systems within the time-domain simulation framework FhSim [1]. This presentation showcases the numerical modelling of a novel membrane enclosure system for open-ocean finfish aquaculture in New Zealand [2, 3]. The focus is on the flexible structure design and mooring optimisation required to withstand offshore loads, as well as the water flow and dissolved oxygen (DO) transport necessary for fish well-being.

## Simulation model

The fish enclosure was modelled as a horizontal fabric cylinder with net-covered ends, stiffened by outer ring structures to maintain its shape (Fig. 1a). The length of the structure ($L$) is 65 m, and the diameter ($D$) is 14 m. A single-point mooring (SPM) system allowed the structure to rotate freely with the flow while submerged below depths strongly affected by waves (Fig. 1b) summarises the main structural parameters used in the present simulations.

## Results and discussions

Time-domain simulations examined the motion of the moored structure in waves and currents, modelling extreme sea states to evaluate structural and mooring reliability under design loads. Fig. 1b shows the structure in an extreme sea state (described by a JONSWAP spectrum): significant wave height $Hs$ = 7.17 m; peak wave period $Tp$ = 11.29 s; current speed $U$ = 0.52 m/s. The predicted vertical displacements of the mooring buoy (2000-L volume) are shown in Fig. 1c, and the corresponding anchor loads are shown in Fig. 1d. Simulations showed that the wave forces acting on the enclosure decrease with increasing submergence, while the anchor loads are also dependent on the buoy size.

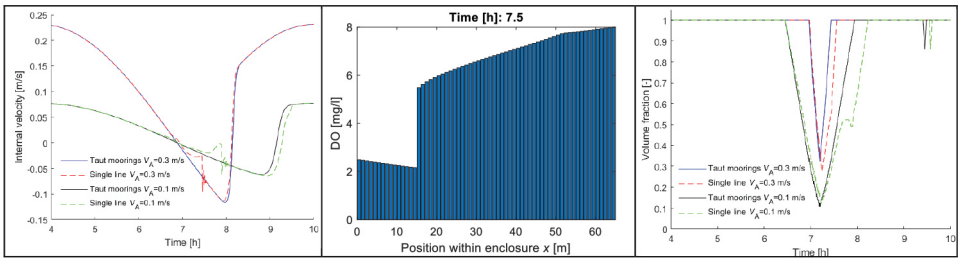


**Fig. 2. Water velocity through the enclosure during the turn of the tide (a), instant DO levels inside the enclosure (b) and time-varying enclosure volume fractions with DO above 60% saturation (c).**

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The model was also used to predict the structure's motion in a harmonic tidal current (with different velocity amplitudes $V_A$) and their combined effect on the water flow through the enclosure (Fig. 2a). Slack (single-line) and taut mooring configurations were considered here. These simulations provided the basis for predicting the DO dynamics within the enclosure considering different water temperatures and fish respiration rates [3]. Since adequate DO levels are crucial for fish welfare, simulations were used to identify periods of reduced DO levels during tidal transitions (Fig. 2b-c). The internal conditions were characterised by evaluating volume fractions with DO levels above 60% saturation, which is considered a threshold for salmon welfare. Results showed that the motion of the structure connected to an SPM system significantly impacts internal oxygen levels, offering insights for optimising the mooring design.

**Conclusion**

The presented case study highlights the benefits of using numerical simulations to support the development of flexible structures for offshore aquaculture. With modifications, the presented numerical framework could also be useful for modelling other aquaculture systems, including conventional net pens.

**Acknowledgements**

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# POSITIVE ANIMAL WELFARE IN FARMED FISH

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## Positive animal welfare as a concept

Positive animal welfare (PAW) is an evolving concept, shifting from minimising suffering to promoting positive experiences. While animal welfare science has traditionally focused on alleviating negative states, there is a growing recognition that the absence of suffering does not automatically equate to good welfare.

The latest effort to clarify meanings and definitions resulted in PAW being described as "the animal flourishing through the experience of predominantly positive mental states and the development of competence and resilience. Positive animal welfare goes beyond ensuring good physical health and the prevention and alleviation of suffering. Positive mental states result from rewarding experiences, including having choices and opportunities to actively pursue goals and achieve desired outcomes, according to species-specific and individual capabilities. Genetic, developmental and experiential factors (e.g. pre-natal, early life, environmental) contribute to individual differences in the ability to achieve positive animal welfare. Positive animal welfare can be assessed using animal-based indicators and can be evaluated over different timescales, thereby contributing to the lifetime picture" [1]. This definition emphasises that PAW goes beyond current established concepts of "good health" or "good welfare" in animals (Fig. 1).

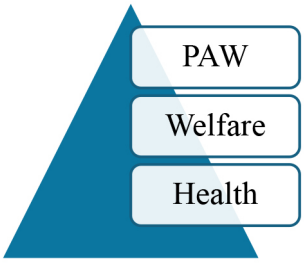


*Fig. 1: A common understanding of clear definitions is important to move concepts from fundamental science theory to applied settings and research*




*Fig. 2: Visit the COST Action LIFT under* www.liftanimalwelfare.eu

## COST Action on PAW in farm animals

Ultimately, PAW aims to improve animal lives by understanding the mechanisms leading to positive experiences [1] and may be a future framework for research, inspiring scientific progress and aligning animal care with societal expectations. This is of particular interest for animal farming, where animal numbers are high, and the purpose of resource production is clear. Therefore, the COST Action "LIFT - Lifting Farm Animal Lives" was initiated in 2022, aiming at laying the foundations for positive animal welfare in research (Fig. 2). This includes creating a clear definition of the concept, identifying valid methods to assess PAW and devising potential indicators of PAW to be used on-farm.

## PAW in farmed fish

Most of the literature around PAW in farmed animals is focused on agriculture and the farming of the most common mammals and birds, while aquaculture and fish are underrepresented, especially given the production volume of global fish farming. This lag can be used as an advantage for aquaculture, as agriculture offers insights into both successful measures and potential failures in including PAW into farming [2]. In order to adapt agricultural practices for measuring, assessing and improving PAW for fish farming, better knowledge of species-specific behaviour and biology is necessary [3]. Therefore, research is needed to identify and monitor species-specific indicators of positive welfare in fish.

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While applied research in aquaculture has made progress in developing welfare monitoring and assessment schemes [4–7], those have a focus on health indicators and include welfare mainly in the form of proxy behavioural indicators. Here, effort is needed to include more welfare aspects, and while fundamental science will allow insights into affect states in fish in the future, QBA (qualitative behavioural assessment) may be a possibility to already now include proxy indicators for PAW in applied science[8–10]. QBA involves observing and quantifying the expressive qualities of an animal's behaviour, using descriptors like 'relaxed', 'inquisitive', or 'energetic', allowing for the inclusion of how behaviours are performed in addition to which behaviours occur, and therefore providing insights into the animal's subjective experiences. However, including an anthropomorphic evaluation of affect states, for example by using the Arousal-Valence Model, the assessment of welfare in fish is likely to become subject to subjectivity and ambivalence[11] which have to be critically reviewed, prevented and counteracted.

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# A COMPREHENSIVE GENETIC STUDY AIMING AT MAPPING QUANTITATIVE TRAIT LOCI AND INITIATING THE FIRST EUROPEAN GENETIC SELECTION PROGRAM FOR THE MEAGRE (*Argyrosomus regius*)

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In the context of the Greek-funded research project MeagreGen, we genetically analyzed more than 900 samples from eleven meagre broodstocks using microsatellite markers, eight of which were from breeders of AVRAMAR SA, as well as three other stocks and their offspring. Meagre is a fast-growing sciaenid species that has recently gained significant interest in aquaculture diversification and the industry's sustainability. The estimated heterozygosity values were generally low (0.40 to 0.84), as was the number of alleles per site (average 3.58). Structure and DAPC analyses suggested that the populations may be optimally grouped into five groups. We analyzed the relatedness results using two selected methods to demonstrate that there is no strong kinship relationship between the stocks. Conversely, we found parent-offspring and/or full-sibling relationships within the stocks (Nousias *et al*., 2021).

We also initiated the first massive production of genetic data in meagre with genomic and transcriptomic sequences. In total, over 38 million base pairs (bp) were sequenced with the ONT MinION platform (long-read sequences), and more than 3 million sequences with an average length of 12,640 bp, providing 54.5× coverage of the genome. Additionally, over 166 million sequences of 150 bp were sequenced using the Illumina platform (short-length and high-fidelity sequences), providing 30.9× coverage of the genome. Moreover, the transcriptome sequencing generated over 100 million sequences that were used for constructing the transcriptome of the species. At the end, the first high-quality reference genome as well as the first gene predictions were delivered for meagre. The genome has a total length of 696 megabases (Mb) and includes 1,012 highly relevant sequences. More than 93% of the genome is included in the 24 largest scaffolds, which correspond to the 24 chromosomes of the species (Papadogiannis *et al*., 2023).

Subsequently, we conducted the first complete genomic evaluation of the species for body weight, length and growth parameters using more than 800 offspring genotyped with a double-digest RAD sequencing protocol. Parentage assignment was performed using SNP markers and a quantitative trait locus (QTL) associated with growth, and explaining approximately 3.25 % of the phenotypic variance was detected in chromosome 17. Using pedigree and genomic relationship matrix, moderate to high heritability estimates along with high genetic/genomic correlations between all studied traits were detected except for the initial body weight. The predicted performance of the breeding values estimation was higher with the genomic than the pedigree relationship matrix for body weight and growth (Oikonomou *et al*., 2025). Lastly, other significant or minor QTLs for head centroid size and skeletal deformities, such as lordosis and pugheadedness, were identified by integrating data from geometric morphometrics, and their use was evaluated as an improvement target in breeding programs. Finally, we propose the application of genomic selection (GS) over MAS, which has advantages over the classical polygenic effects based on pedigree, because it predicts the breeding value for each phenotype with greater accuracy, reduces the generation interval with positive effects on genetic progress, and helps maintain homozygosity at low levels under breeding conditions.

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# VALORISATION OF AQUATIC BIOMASS AND SIDE-STREAMS FOR SUSTAINABLE PACKAGING IN THE BLUE BIOECONOMY: INSIGHTS FROM THE AQUAPACK PROJECT

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## Introduction

Significant amounts of fish and seafood are wasted worldwide due to spoilage and degradation during icing, packaging, storage and transportation after landing (ranging from ≈30% of the initial catching in Latin America, Africa and Europe to 50% for North America and Oceania, according to FAO). Waste at the end of the food supply chain is also substantial in all three regions, with 15-30% of purchases by mass discarded by consumers. Packaging plays a crucial role in adequate preservation and storage of sensitive products. Developing novel and efficient packaging solutions to improve product quality and shelf life and reduce waste while not increasing packaging waste is a major challenge to improve sustainability of food supply chains (Athanasopoulou et al., 2024; Basdeki et al., 2024; Spanou et al., 2024).

Marine biomass contains significant quantities of polysaccharides, proteins and minerals. During the past years, there has been significant interest in natural products obtained from marine organisms, which may provide valuable commodities while at the same time promote health and well-being in humans (Vasilakis et al., 2025). Algae is a promising source for deriving monomers used in plastic production. Both macro- and microalgae are rich primary sources of bioactive compounds and could be used as a source of functional ingredients such as carotenoids, fatty acids, polysaccharides, peptides, vitamins and sterols (Hidhayati et al., 2025; Koletti et al., 2025).

The AQUAPACK project aims to develop active and sustainable packaging materials based on the selection of suitable aquatic biomass (micro- and macro-algae, fish processing side streams and by-catch) with the aim of obtaining appropriate raw materials (carbohydrates, proteins, fatty acids etc.) and biopolymers, to produce biodegradable packaging systems specifically tailored for the food and cosmetic industry. The project is at the interface between research and commercial exploitation and will generate knowledge and innovations to promote growth of the sustainable blue bioeconomy, preserving marine resources and ecosystems.

By valorising underutilized marine resources, AQUAPACK is expected to contribute significantly to reducing reliance on fossil-based plastics, closing nutrient loops, and driving innovation through bio-based packaging alternatives.

## Materials and methods

To turn AQUAPACK objectives into practice, trans-regional cooperation is needed to create a strategic partnership between academia and industry, to ensure resource availability, facilitate knowledge exchange and harmonize policies. AQUAPACK project brings together 15 partners (8 academic and 7 commercial) located in 9 EU countries (Greece, Portugal, Italy, France, Cyprus, Spain, Croatia, United Kingdom and Netherlands). The proposed research work plan is organized into 3 complementary and multi-disciplinary workflows, including: (1) Customized packaging technologies based on aquatic biomass, (2) Development and evaluation of novel packaged food and cosmetic prototypes, (3) Evaluation of the sustainability and safety of biodegradable packaging systems.

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Suitable aquatic bioresources (micro- and macro-algae biomass, fish and seafood production side-streams and by-catch) and algae growth conditions are selected, with the aim to obtain appropriate bulk raw materials (carbohydrates, proteins, fatty acids, biopolymers etc.) for the development and production of novel sustainable and biodegradable systems. The produced aquatic-based bioactive compounds are screened and characterized for application as active components in active packaging systems. Surface functionalization of biopolymers is carried out using cold plasma, to modify biopolymer wettability, mechanical properties and barrier characteristics and for surface disinfection of packaging materials. Biodegradable, safe and high-quality materials are developed and tested for their efficiency as appropriate packaging systems for sensitive products, including fresh fish.

## Results and Discussion

The AQUAPACK project will develop biodegradable, active packaging materials using aquatic biomass and side-streams, targeting both the food and cosmetics sectors. Two representative outputs will be presented: (i) a biodegradable active packaging film designed to preserve the quality and extend the shelf life of fresh fish and (ii) a compostable rigid container tailored for cosmetic products, offering structural integrity and environmental sustainability. These innovations demonstrate strong potential to reduce plastic waste, maintain product safety, and support a circular bioeconomy, while fostering collaboration between academic and industrial partners.

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# IMPACT OF COOKING METHODOLOGY ON NUTRITIONAL QUALITY OF SENEGALESE SOLE FARMED IN IMTA-RAS SYSTEM

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## Introduction

Recent efforts in aquaculture focus on sustainable systems that maintain productivity while preserving product quality. Recirculating aquaculture systems (RAS) minimize nutrient discharge compared to traditional methods, and when combined with Integrated Multi-Trophic Aquaculture (IMTA), they enhance nutrient efficiency by co-culturing fish with extractive species like macroalgae. *Ulva ohnoi* is a key IMTA species due to its rapid growth and strong nitrogen and phosphorus uptake. Beyond improving water quality, *Ulva* is valued for use in animal feed, ulvans for biomedical applications, and as a carrier for probiotic bacteria such as *Phaeobacter* sp., antagonist of fish pathogens. Integrating *U. ohnoi* and *Phaeobacter* in IMTA-RAS may improve fish resilience and nutritional quality, but little is known about how these benefits persist after cooking. Cooking significantly affects moisture, protein structure, lipid oxidation, and sensory traits such as color and flavor. Steaming and baking (common household methods) differ in their impact on nutrient retention and oxidative stability.

This study examined whether co-culturing Senegalese sole with *U. ohnoi* and *Phaeobacter* in IMTA-RAS improves quality parameter retention during steaming and baking. Key metrics included lipid and fatty acid composition, cholesterol, oxidative status, proximate composition, and fillet color and texture. Potential mechanisms included *Ulva*-derived antioxidants and ulvans acting as natural preservatives, and *Phaeobacter*-mediated microbiome effects slowing down spoilage. By linking sustainable production to post-harvest quality, this research aimed to highlight how IMTA-RAS could deliver fish with enhanced nutritional and sensory attributes while reducing environmental impact.

## Materials and Methods

Three growth experiments were conducted: (1) sole in RAS; (2) integrated sole–*Ulva* in IMTA-RAS; (3) like in experiment 2 with *Phaeobacter* sp. inoculation to water. For each experiment, Senegalese soles (~85 g initial body weight) were reared for 8 weeks each. Water quality was maintained with temperature of 18.5–21 °C, total ammonia < 0.05 mg/L, nitrites < 0.8 mg/L, pH 7.2–8.2, and dissolved oxygen > 65%. *U. ohnoi* was cultivated in three parallel couples of tanks under light/dark conditions alongside three fish tanks. Fish were fed commercial pellets (0.45–0.9% of biomass initially, 250 g/day stabilized) via automatic feeders. After 8 weeks, 54 fish (~120 g final body weight) per experiment were sampled by 2-phenoxyethanol overdose, then divided into 3 treatments: raw, steam (STE), and bake (BAK) (18 samples for each treatment). Fish were measured (length and weight), stored at –80 °C, and thawed at 4 °C before the scheduled analyses. A group of fish was analyzed as raw. the cooking methods were steaming (wrapped in foil over boiling water, at ~100 °C) and baking (180 °C, fan oven). Both continued until 62 °C core temperature was reached. The analyses included biometric measurements, skin and fillet color, cooking loss, texture, moisture, ashes, crude protein total lipids, fatty acids, cholesterol, and oxidative status (conjugated dienes, TBARS). Data were subjected to one-way ANOVA followed by Tukey's multiple comparison test. Significance was set at $p < 0.05$.

## Results and Discussion

Fish provides high-quality protein, long-chain n-3 polyunsaturated fatty acids (PUFAs), and essential micronutrients. High intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in human diet is linked to improved lipid profiles and reduced inflammation, underscoring fish's value in a healthy diet for humans. Cooking, however, can alter/modify the fish nutritional quality [1]. Cooking significantly reduced texture ($p<0.0001$) in all samples regardless of the experiment. For instance, in RAS fish, hardness dropped from 10.01 N (RAW) to 4.16 N (STE) and 4.23 N (BAK), while IMTA-RAS (without *Phaeobacter* sp.) samples showed similar trends, falling from 9.72 N (RAW) to 3.54 N (STE) and 3.37 N (BAK).Color also changed after cooking: lightness ($L^*$) increased in all samples, especially in the STE ones, while redness ($a^*$) rose more in BAK than in STE samples, likely due to Maillard reaction. Total lipid content declined after cooking ($p<0.0001$), with the highest losses in BAK, reflecting a greater dehydration. Both methods improved the PUFA/SFA ratio and n-3 PUFA incidence in fatty

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acid profile compared to RAW, with BAK showing a slightly stronger effect. Palmitic acid (C16:0) was the main saturated fatty acid, while oleic acid (C18:1n-9) dominated the monounsaturated fatty acids group. PUFAs, especially of the n-3 and n-6 series, were preserved; DHA was the predominant n-3 fatty acid (9.66–16.19%), followed by EPA (2.18–2.34%). Cooking increased the EPA+DHA content across all three experiments (with a minimum incidence of 15.60%), with STE reaching 18.53% in the Experiment 3. The n-3/n-6 ratio also improved in fish belonging to Experiment 3, reaching >2.0, a value in agreement with the human recommended dietary values. Cholesterol levels rose after cooking due to water loss associated with the thermal treatment, more in BAK (85.49 mg/100g) than in STE (76.60 mg/100g). Oxidation markers showed minimal changes: conjugated dienes decreased slightly with cooking, whilst TBARS values remained low (<0.2 mg MDA/100g), indicating an excellent lipid stability regardless of the cooking method. As fish might have eaten fragments of *U. ohnoi*'s blades that accidently passed to their tanks, algal antioxidant compounds may have further limited oxidation in IMTA-RAS fish. Moisture content decreased significantly ($p<0.0001$) due to thermal processing, especially in IMTA-RAS with *Phaeobacter* sp. (Exp 3). On the contrary, protein levels rose in cooked samples due to water loss, while ash content showed minor, inconsistent increases. However, baking generally caused more pronounced nutrient concentration than steaming.

Overall, both cooking methods altered texture and composition but improved the nutritional quality of the lipid profile, especially by enhancing n-3 fatty acid incidence and improving the PUFA/SFA ratio. IMTA-RAS systems, particularly with *Phaeobacter* sp. (Exp 3), maintained or improved fatty acid ratios and oxidation stability without compromising nutritional value, representing a sustainable alternative to conventional farming systems. The prevalence of DHA over EPA and high EPA+DHA content confirmed *S. senegalensis* as a valuable source of health-promoting fatty acids. However, further statistical comparisons between systems and cooking methods are recommended to confirm these findings.

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# MODELING STAKEHOLDER OPINION DYNAMICS IN GLOBAL AQUACULTURE: AN EMPIRICALLY-GROUNDED AGENT-BASED FRAMEWORK

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The rapid growth of aquaculture has increased conflicts among stakeholders with various assessments of environmental risks, economic returns, and social fairness. Collective analyses do not cover the micro-level processes through which belief revisions and social influences shape governance outcomes. We introduced an empirically parameterised agent-based model that simulates opinion dynamics within the multilayer social–ecological system of aquaculture. A global dataset of media coverage on aquaculture, represented eight stakeholder classes: producers, consumers, government officials, industry leaders, local communities, non-governmental organisations, researchers each holding Bayesian belief states updated by bounded confidence, Bayesian learning, and ideology-conditioned confirmation bias. Stakeholder interactions are designed to occur across various networks, with each layer representing empirical co-occurrence, ideological similarity, and geographical proximity whereas connections among stakeholders evolve through preferential connections moderated by similarity thresholds. Media effects were endogenized with a three-cut ordered probit that maps ideology and topic importance to stochastic sentiment shocks. The model can potentially test policies for interventions such as ideology-targeted outreach, adaptive licencing, and broker-facilitated dialogue with the integration of media sentiment scores, agents, and dynamic networks. This model also aims to demonstrate how data-driven agent-based models can reveal adaptive governance dynamics and inform evidence-based strategies for socially acceptable and environmentally sustainable aquaculture development.

# EMISSION REGULATION – PROPOSED SEA LICE QUOTAS IN NORWEGIAN AQUACULTURE

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In salmon aquaculture sea lice is an externality that contributes to poor welfare of farmed salmon and wild salmon mortality. Sea lice is one of the main factors limiting growth in global salmon production.

In Norway the government has tried to regulate sea lice externalities to wild salmon through a traffic light system (TLS). This system employs several methods to estimate the relationship between sea lice populations in aquaculture farms and sea lice induced mortality of wild salmon smolt migrating from rivers to the open ocean. The Norwegian coastline is divided into 13 production areas. In each production area aquaculture farms are allowed to increase production if the estimated mortality of wild smolt is below 10%, but have to reduce production if mortality is above 30%. The collective reward/punishment system in TLS is assessed to not give salmon firms sufficiently strong incentives to mitigate sea lice externalities.

In April 2025 the Norwegian government proposed a sea lice quota system to replace the collective mechanism in the TLS. The government proposes that the total sea lice quota for a production area is set equal to a level corresponding to 10% sea lice induced mortality. The quota is going to be divided between firms, probably corresponding to their allocated salmon production quota.

The dominant conventional open cage farm technology allows sea lice to migrate in and out of farms. This paper analyzes what levels of investments are necessary in alternative 'low lice' and 'zero lice' production systems to satisfy lice quotas. For each possible sea lice quota level we estimate the production technology combinations of (1) 'low lice' deep sea cages, (2) 'zero lice' sea based closed containment systems and (3) land based closed systems that can satisfy the quota with the highest net present value (NPV). Investment cost per kg of salmon produced increases substantially from deep sea systems via closed containment to land based systems. Furthermore, the production share of each production technology is constrained by nature, government regulations, etc.

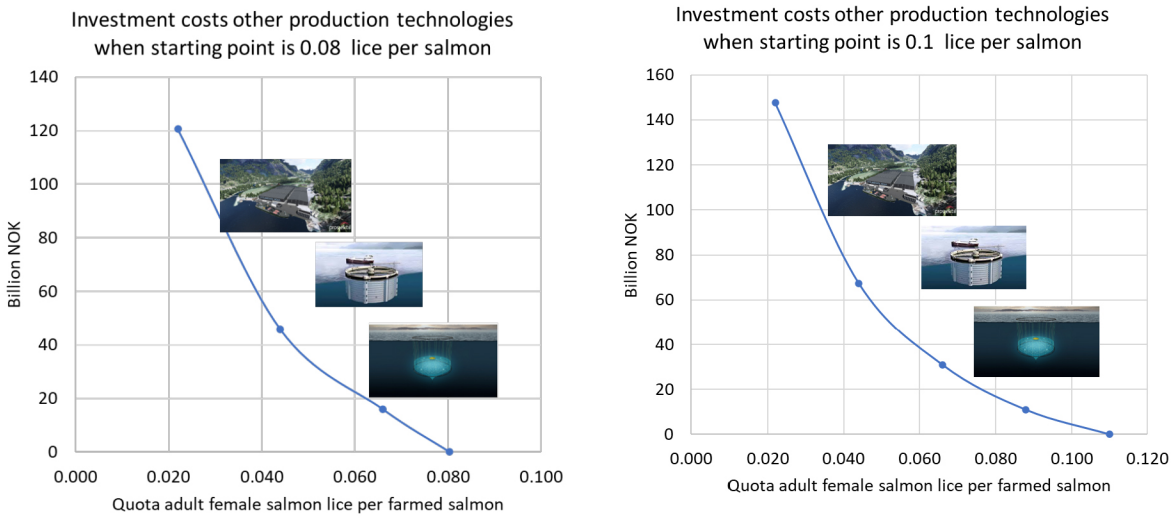


**Figure 1. Estimated investments required to maintain production of 1.6 million metric tonnes (1EUR=12 NOK)**

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Figure 1 shows the estimated investment curves maximizing NPV for different sea lice quota levels and different starting points for sea lice prevalence. It turns out that investments may be in an order of magnitude that will require a multi-year investment horizon, consuming much of the after-tax cash flow of the industry. Future after-tax cash flows in Norwegian salmon aquaculture may be 1-2 billion EUR each year, while the total investment budget may be in the order of 10 billion EUR. As indicated by Figure 1, total investments are highly sensitive to the quota levels. Required investments increase exponentially with a stricter quota, as an increasing share of production must be allocated to the more capital-intensive closed systems in sea and on land.
The effects of sea lice quota design on investment decisions are also analyzed. This includes the transition from TLS to a quota system. An immediate introduction of strict quotas will not be possible to accommodate in several production areas, due to constraints on financial capital, supplier industry capacities and government regulatory capacities. Hence, it is necessary to have a transition period with a sufficient time horizon allowing for sufficient investments and learning. The sea quota can in principle be changed over time due to changes in sea temperature, model estimates etc. We discuss constraints on quota changes, given challenges in adapting to stricter quotas.

Sea lice is also an externality between farms. We analyze the collective investment problem in a production area, as several firms must jointly invest to satisfy quota levels.

A new social contract may be necessary to enable a sustainable path to sustainable aquaculture, with the following elements:

- A sufficient time horizon for investments and adaptation, probably at least ten years.
- Salmon industry commitments to investments in the order of several billion EUR.
- Salmon companies in each production area commit to each other.
- Government commits to predictability with respect to (a) after-tax cash flow available for investments, and (2) administrative processes that enable investment projects.

It is reasonable to argue that a social contract enabling sufficient investments will require a broad political consensus to be credible.

# EFFECTS OF ONION (*Allium cepa*) IN FEED ON BLOOD PARAMETERS OF CATFISH AND AQUA-FARM WATER QUALITY

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Feed is an important factor in aquaculture with the individual ingredients being significant to the physiology of fish and to the condition of culture water. This study therefore investigated the effects of including onion at 0%, 1.02%, 2.05% and 3.07% in fish feed on the haematology of *Clarias gariepinus* and on water quality. The haematological parameters evaluated included red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), haemoglobin content, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Furthermore, cholesterol and protein were determined using spectrophotometric-enzymatic and Bradford methods respectively. Albumin and glucose were determined using kits that detect albumin and glucose-oxidase reaction respectively. Globulin was calculated by subtracting the measured albumin value from the total protein. Water quality parameters evaluated were temperature, dissolved oxygen and pH

There was significant increase ($P<0.05$) in RBC, PCV and Hb but decrease in WBC in the fish fed diet containing onion (Table 1.0). The decrease in WBC became significant ($P<0.05$ when onion was included at 2.05%. However the value of the WBC spiked when the onion concentration was 3.07%. There was no significant difference in the value of MCV at the studied concentrations. The increase in MCH due to onion in feed became significant ($P<0.05$) at 2.05%. Value of MCHC followed the same trend as MCH (Table 2.0). There was decrease in cholesterol and glucose due to onion in feed (Figure 1.0). There was increase in total protein and albumin but decrease in globulin due to onion in the diets (Figure 2. 0). Lowest globulin was observed when onion was included at 2.05%. There was no significant difference ($P>0.05$) in the water quality parameters studied among the treatments (Table 3.0). Results from the study suggest 2.05% as a preferred inclusion level of onion in feed for African catfish.

Table 1.0: Values of some haematology parameters of *Clarias gariepinus* fed diets containing different percentage levels of onion

| Onion in diet | RBC Cells/μL | PCV % | Hb g/L | WBC Cells/μL |
|---|---|---|---|---|
| 0% | $3.67\pm 0.07^{c}$ | $25.8 \pm 0.91^{c}$ | $9.2 \pm 0.2^{b}$ | $9.44 \pm 0.66^{a}$ |
| 1.02% | $3.97 \pm 0.12^{b}$ | $27.8 \pm 0.80^{b}$ | $9.96 \pm 0.47^{b}$ | $9.38 \pm 0.30^{a}$ |
| 2.05% | $4.15 \pm 0.06^{a}$ | $28.9 \pm 1.00^{b}$ | $11.4 \pm 0.45^{a}$ | $8.69 \pm 0.22^{b}$ |
| 3.07% | $4.30 \pm 0.11^{a}$ | $30.9 \pm 0.51^{a}$ | $11.6 \pm 0.30^{a}$ | $9.17 \pm 0.98^{b}$ |
| Test | * | * | * | * |

Mean with the same superscript letter in the same row are not significantly different at $P<0.05$

Table 2.0: Values of MCV, MCH and MCHC in *Clarias gariepinus* fed diets containing different percentage levels of onion

| Onion in diet | MCV fL | MCH Pg | MCHC g/L |
|---|---|---|---|
| 0% | $70.16 \pm 1.94$ | $25.01 \pm 0.55^{b}$ | $35.66 \pm 0.51^{c}$ |
| 1.02% | $69.96 \pm 0.14$ | $25.08 \pm 0.83^{b}$ | $35.84 \pm 1.15^{c}$ |
| 2.05% | $69.72 \pm 1.33$ | $27.43 \pm 0.63^{a}$ | $39.34 \pm 0.28^{a}$ |
| 3.07% | $71.88 \pm 0.68$ | $27.10 \pm 0.52^{a}$ | $37.71\pm 0.60^{b}$ |
| Test | Ns | * | * |

Mean with the same superscript letter in the same row are not significantly different at $P<0.05$

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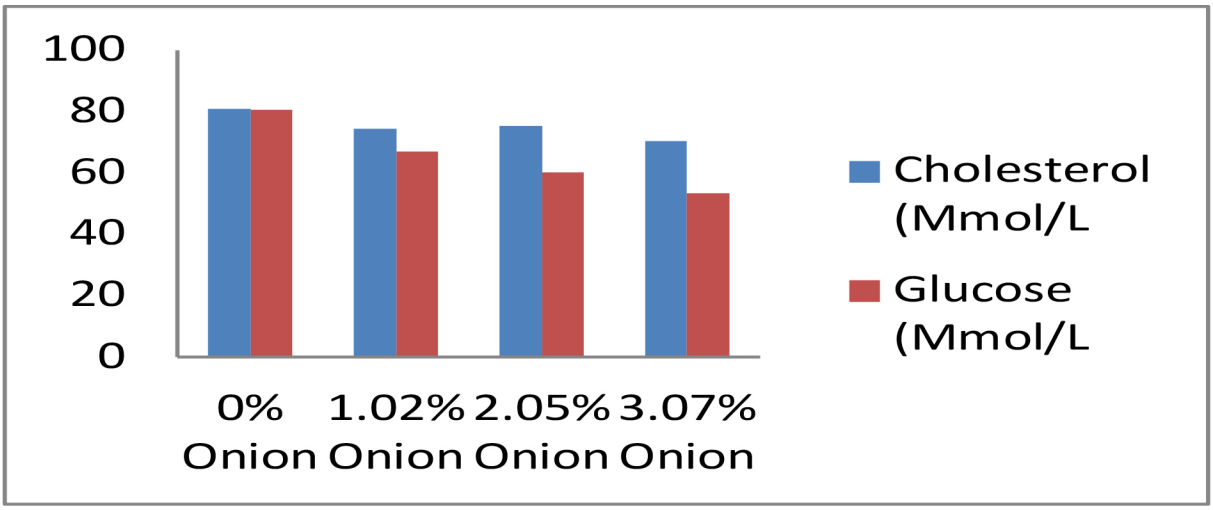


Figure 1.0: Effect of onion in feed on level of cholesterol and glucose in blood of catfish

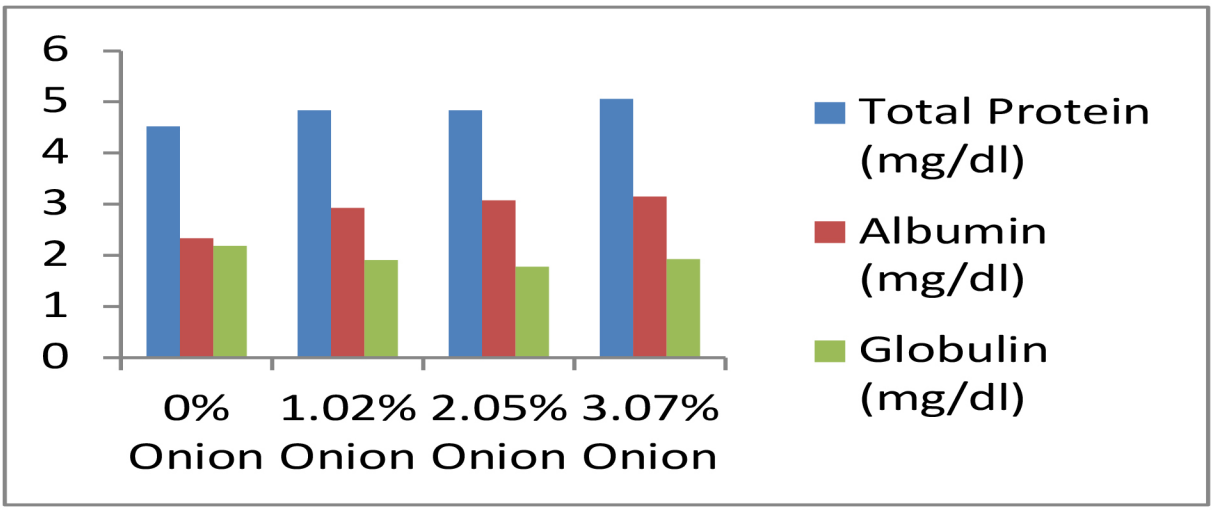


Figure 2.0: Effect of onion in feed on level of protein, albumin and globulin in blood of catfish

Table 3.0: Impacts of treatments on water quality

| | pH | DO (mg/L) | Temperature (°C) |
|---|---|---|---|
| 0% | 5.04 | 18.75 | 315 |
| 1.02% | 5.05 | 18.50 | 31.70 |
| 2.05% | 5.24% | 18.20 | 31.70 |
| 3.07% | 5.43% | 18.00 | 31.50 |
| Test | ns | ns | ns |

# CONCENTRATED NUTRIENTS RECOVERED FROM EFFLUENT WATER FROM RECIRCULATING AQUACULTURE SYSTEM (RAS) USED AS FERTILIZER IN A HYDROPONIC FARM

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## Introduction

The transition to a circular bioeconomy hinges on innovative green technologies able to recycle waste streams into reusable components. This study investigates a pilot-scale system for nutrient recovery from recirculating aquaculture system (RAS) effluent water employing membrane technologies for fertilizer production. The innovation could provide the Norwegian aquaculture sector with an alternative to discarding RAS effluent water, potentially making the industry more sustainable by reducing eutrophication and the need for wastewater treatment. Moreover, the production of a novel recirculated fertilizer product from RAS effluent water might alleviate the dependency on mineral fertilizer across agricultural sectors. For instance, Norwegian agriculture consumed 87,000 tons of nitrogen and 7,000 tons of phosphorus mineral fertilizer in 2023 [1]. This dependency poses significant challenges due to the energy-intensive production of nitrogen fertilizers, which represents 2 % of the global energy consumption and the emission of 450 mT $CO_2$ annually [2].

The primary goals of the study were to evaluate the performance of the water treatment system when producing a concentrate intended as fertilizer from RAS effluent water, to assess the storage stability of the produced fertilizer with and without pasteurization, and to assess the concentrates suitability as a fertilizer. By measuring the input and output streams of the water treatment system over time, the study aimed to determine system performance and compare the composition of the resulting concentrate to plant nutrient requirements. Additionally, the chemical and microbiological storage stability of pasteurized concentrate was monitored for six months and compared to an unpasteurized control. The concentrates suitability as a fertilizer was tested through plant cultivation trials in a hydroponic farm.

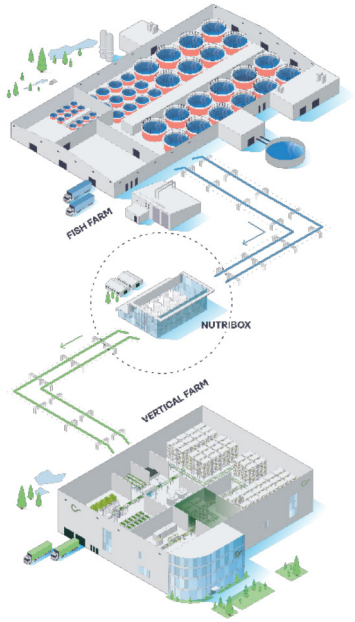


***Figure 1.*** *Effluent water from RAS is processed through a water treatment unit (Nutribox"), to produce clean water that can be recirculated back to the RAS, and a concentrate used as fertilizer for plant cultivation in a vertical farm.*

## Materials and methods

A pilot system consisting of a research scale RAS facility, a water treatment system, and a vertical farm was built and operated for the project duration of three years, located at the research station of NIBIO Landvik, Norway. The RAS system consisted of two 1,000 L fish rearing tanks with *Salmo salar* grown at a density between 70 – 100 kg/m$^3$, a moving bed biofilter, $CO_2$ scrubber, oxygenation, pH regulation and a hydrocyclone and drum filter for sludge removal. All RAS effluent water of 900 – 1,200 L/ week was processed in batches of 300 L through the water treatment system to produce a nutrient rich concentrate and clean permeate. The storage stability of pasteurized and unpasteurized concentrate was assessed through a six-month shelf-life study monitoring chemical and microbiological changes. Meanwhile, the concentrates potential as a fertilizer was assessed through plant cultivation studies in a 6.72 m$^2$ hydroponic vertical farm.

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**Results and discussion**

The water treatment system used RAS effluent water to produce a concentrate with a high element ratio of the plant macro nutrients nitrate and potassium. However, the extract was low in phosphorous due to the low contents of water soluble phosphate in the RAS effluent water. Preliminary results from the hydroponic cultivation trials indicated that RAS concentrate enriched with mineral fertilizer achieve plant yields comparable to the mineral fertilizer control treatment. In the shelf-life study the RAS concentrate was found to be microbially and chemically stable for at least six months regardless of pasteurization.

The study show that concentrated RAS effluent water can be used as a fertilizer in plant cultivation, which have the potential to alleviate the consumption of mineral fertilizer while simultaneously decreasing the eutrophication potential of commercial RAS systems by collecting and using discharged nutrients. In future studies, efforts should be made to concentrate the fertilizer further to make distribution to a global marked more feasible, asses the economic and environmental feasibility of a scaled-up system and investigating the impact on fish-health when recycling water cleaned by the developed water treatment system back to the RAS.

This research is part of the IPN Nutricycle project, and is funded by the Research Council of Norway (grant number # 336971)

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# UPSCALING RESOLUTION IN FISH ACOUSTIC TELEMETRY

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**Introduction**

Understanding behavior of fish in aquaculture relies on semi-continuous monitoring, yet obtained data are discrete and often irregular. This study investigates the fish acoustic telemetry RMS acceleration measurements as a stochastic stationary process. Unlike in the wild conditions, where fish behavior is highly variable and nonstationary, captive aquaculture environments should offer stability and predictability. The stability is valid under the assumption of *weak stationarity*, where the mean and variance of the process remain constant over time. Such conditions is justifying the use of probabilistic model which preserves the statistical characteristics during interpolation to reconstruct the continuous behavior from discrete samples.

**Dataset and Methods**

The dataset analyzed in this study comprises root mean square (RMS) acceleration values recorded via acoustic telemetry of Atlantic salmon held in captive. The data covers the period of 148 days, with approximately 92,500 values for a single fish. The measurements were taken in non-equidistant intervals ranging from 220 to 380 seconds. The acceleration values range from 0 to 255, with each unit increment corresponding to 0.014 m/s². The acceleration time series is modeled as a causal stochastic system using discrete Markov transition matrices to estimate conditional probabilities between acceleration states. The data passed the tests for weak stationarity (Augmented Dickey-Fuller test), for Markov property (Chapman-Kolmogorov Equation), and for practical differentiability of the autocorrelation function. A Wiener-Levy process was then applied to perform high-resolution interpolation of transition probabilities while preserving the mean and standard deviation of the original distributions.

**Results and Discussion**

Two levels of continuation tests were performed: one on reduced transitions matrix, and one witht 100× finer resolution. The results demonstrated that the RMS acceleration data from captive Atlantic salmon exhibited weak stationarity and practical differentiability, validating its suitability for modeling as a diffusion system. Transition matrices revealed dominant self-transitions, indicating behavioral stability. The application of the Wiener-Levy process, repeated 1,000 times for each transition, enabled fine-resolution interpolation that preserved statistical properties.

These tests confirmed that the system exhibits the necessary smoothness and continuity properties, validating the use of Wiener-Levy diffusion as a tool to simulate realistic interpolated behavior in fish activity.

**Acknowledgment**

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# ENVIRONMENTAL INTERACTIONS AND POSSIBLE SOLUTIONS FOR CARP-DOMINANT POND AQUACULTURE IN HUNGARY

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Carp-dominant pond aquaculture is a traditional and significant sector in Hungary's freshwater fish production, with deep-rooted traditions and significant socio-economic value. However, its environmental interactions - such as nutrient loading, eutrophication, sediment accumulation, and biodiversity impacts - raise ecological concerns. Climate change further exacerbates these issues by altering water availability, increasing temperatures, and intensifying weather extremes, all of which affect pond ecology and fish health. In response, sustainable and adaptive management strategies are urgently needed.

Potential solutions include integrated multi-trophic aquaculture (IMTA), optimized feeding strategies, water quality monitoring, and better sludge and effluent management. From a policy perspective, encouraging data-driven decision-making, farmer training, and the adoption of best management practices (BMPs) will be critical. Strengthening research–industry collaboration and revising subsidy schemes to favor environmentally friendly technologies can support this transition. By addressing both ecological impacts and production efficiency, Hungary can lead the way in developing sustainable, climate-resilient carp pond aquaculture systems.

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# EFFECT OF LIGHT ON PRODUCTION AND BREEDING PARAMETERS OF POND FISH SPECIES

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Light plays a fundamental role in the behaviour, physiology, and growth performance of freshwater fish species. In aquaculture, manipulating light conditions can significantly influence feeding activity, reproduction, stress response, and overall productivity. This study reviews and compares the effects of light intensity, photoperiod, and spectral composition on the production of key species cultivated in Central and Eastern Europe: common carp (*Cyprinus carpio*), Chinese carps (e.g., *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*), northern pike (*Esox lucius*), pikeperch (*Sander lucioperca*), and European wels (*Silurus glanis*).

Carp and Chinese carps show adaptive responses to natural light cycles, but extended daylight hours under controlled conditions have been shown to improve feed intake and growth in juvenile stages. Pike and pikeperch, both predatory species with more sensitive visual systems, require carefully managed low-light environments during larviculture to reduce cannibalism and optimize survival. Light colour (e.g., blue or green wavelengths) can further affect their behaviour and feeding success.

European wels, a nocturnal benthic predator, is especially sensitive to light intensity. Dimming light or maintaining dark conditions during rearing improves welfare and reduces stress-induced aggression. Tailoring light regimes in hatchery and grow-out phases is therefore critical to meet species-specific needs.

Understanding the species-specific light preferences and tolerances offers practical benefits for optimizing production protocols, especially in recirculating aquaculture systems (RAS) and hatcheries. Future research should focus on integrating smart lighting technologies with precision aquaculture to improve fish welfare and yield across diverse freshwater species.

The work was supported by MAHOP_PLUSZ-1.2.1-24-2024-00001 project.

# FROM LAB TO WILD: AN INTEGRATED STRATEGY FOR NURSEHOUND CONSERVATION. OPTIMIZING REINTRODUCTION VIA ENVIRONMENTAL ENRICHMENT AND ASSESSING WILD POPULATIONS WITH POPULATION GENOMICS

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## Introduction

The Small Sharks project aims to establish a foundation for a successful reproduction and reintroduction program for the endangered nursehound (*Scyliorhinus stellaris*).

A core component of this work is to determine whether nursehounds raised in captivity have innate habitat preferences or if their preferences can be altered by 18 months of environmental enrichment (Arechavala *et al.,* 2022). We will test this by conducting an acoustic tracking experiment in the Balearic Islands. The data collected on residency time, dispersion, movement, and survival will be used to test our hypotheses and improve future reintroduction and conservation efforts.

A second objective of the project involves next-generation sequencing (NGS) of nursehounds. This genetic analysis will establish a baseline for continuous monitoring, allowing us to detect changes in population dynamics. This genetic information will also inform conservation and breeding programs designed to promote greater genetic variability, thereby enhancing the resilience of wild populations (Avise, 2008, Ardura *et al.*, 2011).

## Material and methods

An additional network of Thelma-Biotel TBR 800 receivers was installed in the Palma Marine Protected Area, spaced 300 meters apart. This expanded the existing infrastructure of the Balearic Tracking Network (BTN).

For the reintroduction study, a Thelma-Biotel A-HP9L transmitter was surgically implanted into the visceral cavity of each nursehound. Thirty individuals that had been raised with environmental enrichment (e.g., artificial seaweeds, tiles, and shelters) were released at the bottom in the center of the receiver network. One week later, a second group of 30 individuals, raised without environmental enrichment, was released in the same location.

To assess population connectivity, blood, tissue, or fin samples from nursehounds in the Balearic Islands, the Adriatic Sea (Italy and Croatia), the Ionian Sea (Greece), and the Cantabrian Sea (northern Spain) are being analyzed to obtain a SNP panel.

## Results and discussion

The receivers will be retrieved from the sea at the end of September, at which point the data will be analyzed. We already know that one nursehound from the environmentally enriched group was captured just one week after its release. The animal was found 5 miles away and at a depth of 60 meters.

Based on previous findings, our working hypothesis is that animals raised with environmental enrichment are better conditioned to use the shelters in the release area and are therefore less likely to disperse quickly. This is supported by earlier studies in the Balearic Islands (Vanrell et al., 2024) which show that nursehounds raised without this enrichment disperse rapidly.

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The NGS sequencing will be performed in specialized centers with proven expertise in these techniques. The resulting raw data will then undergo **bioinformatics analysis** to identify and generate a comprehensive **SNP panel**. This panel will be used to assess the genetic variability, population structure, and connectivity among the different nursehound populations.

The central hypothesis is that the nursehound population of the Balearic Islands is genetically distinct from the other populations being analyzed. This is based on the species' demersal nature, a preferred depth range that does not exceed 400 meters, and their sessile eggs, which do not contribute to dispersal. The deeper waters surrounding the Balearic Islands are therefore hypothesized to act as a physical and genetic barrier. If our genetic analysis confirms this differentiation, it would have significant implications for conservation, suggesting that this population should be managed as a separate and vulnerable unit to ensure its long-term viability.

## Acknowledgments

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# *IN VITRO* CONTRIBUTION OF MACROPHAGE TO THE INNATE IMMUNE-LIKE RESPONSE AND AUTOPHAGY MODULATION IN RTGILL-W1 CELLS CHALLENGED WITH *Piscirickettsia salmonis*

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## Introduction

*Piscirickettsia salmonis* is the etiological agent of the *Salmonid Rickettsial Septicemia (SRS)*, which induces a systemic infection in fish generating millions of dollars in losses to the salmon industry. The bacterium can enter the host through mucosal surfaces, being the gills one of the primary routes of entry. Additionally, *P. salmonis* can modulate the autophagy process, a non-canonic immune mechanism in gill epithelial cells. In this context, gills are multifunctional tissue that combine physical barrier and immune defenses, where epithelial cells interact with immune cells to protect fish against environmental challenges and pathogens. Preliminary studies conducted by our research group have demonstrated that the gill epithelial cell line of rainbow trout RTgill-W1 can respond to *P. salmonis* infection, by upregulating the expression of pro-inflammatory cytokines and pattern recognition receptors. However, the interplay between macrophages and the epithelial gill cells on mounting an effective immune response against *P. salmonis* and the related autophagy modulation is still unknown. In this context, our study hypothesized that the presence of RTS-11 macrophage cell line in co-culture with RTgill-W1 epithelial cells enhances the immune-like response of RTgill-W1 against *P. salmonis,* by increasing pro-inflammatory cytokine expression and activating autophagy. The main objective was to characterize at the transcriptional and protein levels how the interaction between gill epithelial cells and macrophages interact to cope with *P. salmonis* infection.

## Methodology

To address this, an *in vitro* co-culture of RTgill-W1 cells and RTS-11 macrophages/monocytes cell line was established. The co-culture of RTgill-W1 / RTS-11 was challenged with *P. salmonis* for 24 hours, where the bacteria was used only in the RTgill-W1 area. After 24 hours of challenge, mRNA was extracted from RTgill-W1 cells. The expression of immune-related genes (immune receptors, pro-inflammatory cytokines and antimicrobial peptides) and autophagy-related genes was evaluated through real time qPCR, along with the activation of autophagy through changes in the protein content of LC3-II/LC3-I ratio. Additionally, an ELISA assay was performed to evaluate TNFα and IL-1β levels in the RTS11 media.

## Results

The results show that *P. salmonis* infection enhances the immune response of RTgill-W1 cells under co-culture conditions compared to a non-infected co-culture through the up-regulation of pro-inflammatory cytokines TNFα, IL-1β and IL-8 and the modulation of different pattern recognition receptors (PRRs) like NOD1. Additionally, the antimicrobial peptide (AMP) hepcidin was up-regulated in the presence of macrophages. In RTS-11 media, TNFα levels was higher in contrast with non-infected group. Interestingly, autophagy was downregulated under co-culture conditions at protein level, suggesting that the presence of macrophages might inhibit the activation of this process through the release of certain cytokines. However, further experiments are required to analyze and determine how macrophages interfere with autophagy activation. Our preliminary results suggest that the presence of macrophages enhances the immune response of gill epithelial cells, whereas autophagy appears to be reduced. This co-culture model provides a valuable platform for elucidating the cellular communication mechanisms between epithelial and immune cells in fish.

# IN VIVO STUDY OF THE ANTI-INFLAMMATORY AND INMUNOMODULATORY EFFECTS OF *Ulva rigida*-DERIVED POLYSACCHARIDES ON TISSUE REGENERATION IN *Danio rerio*

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The search for new pro-regenerative drugs focuses on substances of a variety of origins with biological activities that include immunomodulation, pro-angiogenesis, antioxidation, or anti-inflammation [1,2]. In this field, *Danio rerio* has become a well-established experimental model for drug evaluation due to its genetic, reproductive and developmental advantages.

In this communication, we investigate the anti-inflammatory and immunomodulatory effects of polysaccharides extracted from *Ulva rigida* (Ulvans) during *Danio rerio* fin regeneration. Our aim is to assess the regulative potential of these extracts over wound healing, blastema formation and regeneration outgrowth to evaluate potential applications in aquaculture.

These effects were studied over three-month zebrafish adults previously fed for two months with Ulvan-containing diets under a constant feeding regime. At 70dpf, the caudal fins of experimental and control zebrafish were amputated to assess regeneration of this organ over a 30-day period. During this procedure, stocking density, size heterogeneity, and food particle size were controlled. Body length, weight and fin anatomy was measured during this process and morphometrical results adjusted to mathematical growth models. In vivo fluorescence probes for oxidative, mitochondrial or endoplasmic reticulum stress were also studied under confocal and fluorescence microscopies.

Preliminary results suggest a slight amelioration of the morphometrical parameters of fin regenerates of fishes fed with the experimental feeds. This study is part of a global comparison of the biological effects of functional feeds over zebrafish and other fish species of economic interest. This study has been funded by a **N**extGenerationEU grant, European Union (PCM-00035, Marine Sciences).

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# ENHANCING TRACEABILITY IN LAND-BASED AQUACULTURE

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## Introduction

To ensure sustainable aquaculture practices and keep end consumers well informed, effective traceability tools are essential. As part of the SEA2SEE project (https://sea2see.eu/), an innovative end-to-end blockchain model has been developed to capture data across the entire seafood value chain. The aquaculture value chain involves multiple stakeholders, including enginnering companies that support farming operations. In this context, Landing Aquaculture, together with SEAentia, has been demonstrating how IoT technology can be applied in indoor recirculating aquaculture systems (RAS) to provide real-time traceability of both the farming environment and the fish production process.

## Materials & Methods

For the demontration case, a state-of-the-art RAS (Fig1), previously designed by Landing Aquaculture, was used to simulate the commercial production of meagre (*Argyrosomus regius*) at SEAentia in Peniche, Portugal. The RAS (~60 $m^3$) consists of eight tanks (2 fingerling, 3 on-growing, 2 grow-out, and 1 purging tank), all connected to a centralized water treatment unit. Designed to operate under a feed load of approximately 20 kg/day with 95% daily water recirculation, the system ensures optimal water quality through mechanical and biological filtration, gas exchange, and temperature control. Solids are removed using a drum filter (CM Aqua F2-1T) and protein skimmer with ozone injection (Clarity CL10); ammonia is reduced in a moving-bed bioreactor (4.5 $m^3$ RK plastic bio media; 750m2/m3); carbon dioxide is stripped via a degasser (0.8 $m^2$); dissolved oxygen is maintained with a low-head oxygen cone (75-100 $m^3$/h); and temperature is regulated using a heat pump (DM-Vario). An OxyGuard monitoring system enables real-time data collection and alerts for deviations, with all water quality data stored in the SmartWater Cloud management software to support bi-weekly analysis and integration into the blockchain traceability model. Each fish batch is eventually linked to a QR code, providing consumers with transparent, easy-to-read information on the fish's life from hatch to harvest.

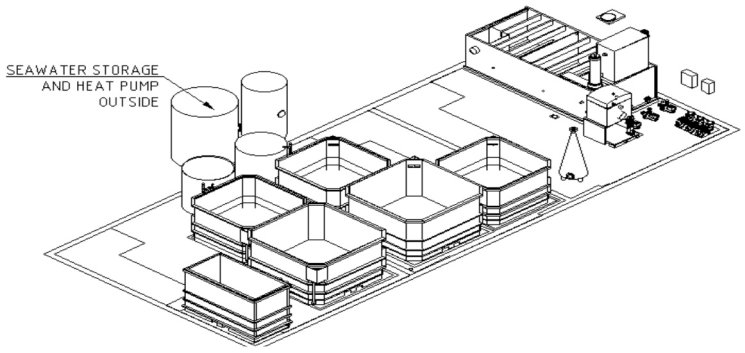


Fig1. RAS layout featuring its key components.

*(Continued on next page)*

## Results & Discussion

With the implemented system design, water quality was consistently maintained at optimal levels for the cultured species, supporting a stable annual production of 5-7 metric tonnes. The real-time monitoring system provided SEAentia with continuous insights into culture conditions, easing farm management by offering full control over the farming environment. Additionally, the continuous digital logging of water quality data created a reliable operational record, supporting SEAentia's qualification for certifications such as the Aquaculture Stewardship Council (ASC) and Best Aquaculture Practices (BAP).

By integrating this data into the blockchain model, stakeholders across the value chain gain access to transparent, verifiable information about production conditions. This apporach not only prevents data loss or miscommunication, but also strengthens consumer trust. This pilot serves as a blueprint for future large-scale applications aimed at increasing transparency in seafood supply chains.

## Funding

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# BOOSTING GROWTH: ENVIRONMENTAL ENRICHMENT'S IMPACT ON CAPTIVE-BRED *Scyliorhinus stellaris*

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## Introduction

Sharks play a fundamental role in maintaining the ecological balance of marine ecosystems (Motivarash *et al*., 2020). Despite their ecological importance, sharks are increasingly at risk due to the widespread and accelerating global decline in the variety and abundance of biodiversity. One example is large-spotted dogfish (*Scyliorhinus stellaris)*, which is listed as vulnerable species due to ongoing population declines (IUCN SSC Shark Specialist Group, 2010). In the Balearic Islands, a restocking program has been established to raise large-spotted dogfish in captivity with the goal of reintroducing them into their natural habitat. Ensuring the well-being of these sharks during their time in captivity is crucial for the success of this effort. This study investigates the effect of different types of environmental enrichment on the growth of this species. To evaluate growth performance, individuals were maintained under experimental conditions with varying habitat complexities over an eight-month period. Enhancing enclosure complexity through environmental enrichment can create a more natural setting that promotes increased foraging behavior (Näslund & Johnsson, 2014).

## Material and methods

A total of 45 large-spotted dogfish born in captivity were tagged with microchips and randomly divided into three groups of 15 individuals, each kept in a 1000 L tank with oxygen and temperature control maintained at 18ºC. Each tank featured a different type of structural environmental enrichment: artificial seaweed (TL1) to simulate natural vegetation, ceramic tiles (TL2) providing structural complexity, and a control group without any environmental enrichment (TL3). The sharks were fed *ad libitum* three times per week, with the feed amount calculated at 5% of their live body weight (Janse, 2003). The diet included fresh oily fish (anchovies), hake, crustaceans, and squid.

Following standard dosing protocols to ensure minimal stress and rapid recovery, the individuals were anesthetized monthly using MS-222 (tricaine methanesulfonate) in order to measure parameters such as length and weight.

The results were analyzed using R statistical software with ANOVA test.

| | Df | Sum Sq | Mean Sq | F value | Pr (>F) |
|---|---|---|---|---|---|
| Tanc | 2 | 500 | 249.77 | 3.785 | 0.0238* |
| Residuals | 292 | 19627 | 65.98 | | |

Signif. Codes: ns not significant; *** <0.001; ** < 0.01; * <0.05

Table 1: Analysis of variance (ANOVA)

*(Continued on next page)*

## Results and discussion

In TL1, individuals had an average initial weight of 134.9 g (SD = 42.7 g) and an initial length of 31.78 cm (SD = 2.53 cm). At the end of the experiment, TL1 individuals reached a mean weight of 828.82 g (SD = 89.22 g) and a mean length of 56.67 cm (SD = 1.71 cm). In TL2, the initial average weight was 118.24 g (SD = 37.49 g), and the initial length was 30.89 cm (SD = 2.82 cm). Final values in TL2 reached 712.72 g in weight (SD = 84.08 g) and 53.73 cm in length (SD = 2.23 cm). Finally, TL3 individuals started with an average weight of 125.82 g (SD = 35.40 g) and a length of 31.35 cm (SD = 2.81 cm), reaching a final average weight of 707.97 g (SD = 104.27 g) and a length of 54.46 cm (SD = 2.52 cm).

The ANOVA analysis revealed a significant overall effect, with an F-value of 3.78 and a p-value of 0.0238, indicating that there are statistically significant differences among TL1, TL2, and TL3 (Table 1).

Several environmental factors may help explain these growth differences among groups. Observations during the trial suggested that individuals in TL1 and TL3 appeared to have easier access to food compared to those in TL2. Additionally, both TL1 and TL2 had more hiding places than TL3, which may have influenced stress levels and consequently, growth. These differences in environmental complexity and food availability may have contributed to the observed variation in final size among the treatment groups.

Enrichment strategies vary depending on species, age, sex, and reproductive condition, and should ideally be adapted to the specific needs of each individual animal rather than applying a universal approach across populations or species. Furthermore, enrichment has been shown to reduce stress in captive animals and promote natural behaviors (Corcoran, 2015).

In summary, our results suggest that environmental enrichment plays a role in the growth of *Scyliorhinus stellaris*. Artificial seaweed (TL1) appeared to promote the greatest growth in both weight and length, compared to ceramic tiles (TL2), while the control group (TL3) showed intermediate outcomes that were not significantly different from either. Therefore, we conclude that environmental enrichment can positively contribute to the welfare and development of *Scyliorhinus stellaris* in captivity, supporting better growth under enriched conditions.

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# WET AND DRY MECHANICAL FRACTIONATION OF *Tenebrio molitor* LARVAE AND THE PROXIMATE COMPOSITION AND *IN VITRO* DIGESTIBILITY OF THE OBTAINED FRACTIONS

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## Introduction

Insects are a promising alternative protein source for aquafeed since they have an adequate nutritional profile, with a high protein content, that can account for more than half of the weight of the dried larvae (Rumpold and Schlüter, 2013). Insect meals, apart from protein, also contain fat and chitin, which can be separated and further utilized. To obtain these meals, the insect must undergo several processing steps, which may include slaughter, drying, and certain types of fractionations, such as defatting (Ojha et al., 2021). These processing steps affect different parameters in the obtained meals, such as nutritional, sensory, and hygienic-sanitary quality (Varga et al., 2024). Mechanical pressing is a relatively cheap, scalable, and economical technique. The fractionation of insects by mechanical dry pressing is frequently applied to eliminate partially or totally the insect fat, while wet pressing is intended to obtain a protein fraction composed mainly of the insect hemocoele and another fraction composed primarily of the insect exoskeleton, rich in chitin. In this study, we evaluated the possible use of each of these fractions as protein-rich meals in aquafeed.

## Materials and methods

*Tenebrio molitor* (TM) larvae were purchased from Artroposfera (Toledo, Spain). Larvae were sacrificed by immersion in liquid nitrogen and stored at -20 °C until further processing. TM larvae were fractionated in a mechanical press at 135 °C. The larvae were freeze-dried (Telstar Lyoquest -55 °C, Barcelona, Spain) prior to the dry-pressing process; a part of the whole dried TM larvae was ground (COM) and stored at -20 °C. DPD fraction was obtained by hot-pressing the dry TM larvae. Wet fractionation was carried out in similar conditions as the dry-pressing process, obtaining a chitinous fraction (WPE) and a fraction consisting mainly of the larval hemocoele (WPH). WPE and WPH fractions were freeze-dried and stored at -20 °C.

Proximate composition of TM meals was carried out according to the methods described by the Association of Official Analytical Chemists (AOAC, 2005). The amino acid profile composition was analyzed with a Biochrom 30+ amino acid analyzer (Biochrom LTD., Cambridge, UK). *In vitro* protein digestion was performed simulating the gastric and intestine steps of the digestive process, following the modified method described by Varga et al. (2024). The protein digestibility of TM meal was evaluated using the enzyme extracts from the stomach and intestine of *Sparus aurata*, and the degree of hydrolysis of gastric phase (DA) and intestinal phase (DB) was expressed as g of -$NH_2$ released with respect to the total -$NH_2$ in the meal.

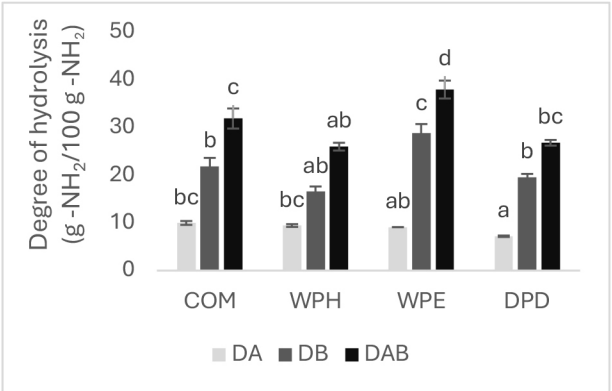


**Figure 1.** Degree of hydrolysis of the obtained fractions. DA: degree of hydrolysis of gastric phase; DB: degree of hydrolysis of intestinal phase; DAB: total degree of hydrolysis. Different letters indicate significant differences (P<0.05).

*(Continued on next page)*

**Results and discussion**

The dry-pressing process yielded 245.2 g of DPD meal and 22.7 g of fat from 1 kg of fresh TM, reaching 845.8 and 78.30 g of DPD and fat, respectively, when calculating with the dry starting material. The wet-pressing process resulted in 124.5 g of WPE (45 % of dry matter) and 150.4 g (55 % of dry matter) of WPH for each kg of fresh TM used. As for the proximal composition, the protein content calculated from the nitrogen content obtained by Kjeldahl analysis results in a significant increase in crude protein for the WPE and DPD meals compared to the unfractionated insect (COM). The crude fat content was significantly decreased for the WPE and DPD meals with respect to COM, while the WPH fraction accumulated most of the fat resulting from wet pressing. Due to the high content of non-protein nitrogen in insect meals, the Kjeldahl analysis and the choice of the nitrogen conversion factor can lead to errors in the calculation of the protein content. The total amino acid content calculated by HPLC showed that the protein content in WPE accounted for more than two-thirds of the dry weight of the meal.

As shown in Figure 1, the hydrolysis degree results showed that the gastric digestion (DA) resulted in similar values except for DPD, which had a significantly reduced degree of hydrolysis. Furthermore, in the intestinal phase (DB), the degree of hydrolysis was clearly seen to be significantly increased and reaches its maximum value for WPE with respect to the rest of the treatments. However, when it comes to the total *in vitro* protein digestibility, this effect lessens.

Results showed that the obtained fractions have high protein levels, satisfactory amino acid profiles, and good *in vitro* amino acid digestibility. Thus, either the complete insect may be directly included in the diet, or DPD may be applied when the separation of fat is crucial. As for WPH, Kang et al. (2023) have previously suggested its use for human consumption as it had excellent properties as 3D printable food. In the same study, WPE was discarded, which, based on the results of the present work, may be destined to aquafeed as it is a more promising protein source than it was previously believed. The remaining chitin content can be either separated for further use; or can remain part of the meal, as several authors confirm its beneficial effects in aquafeed.

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# CIRCALGAE: UNLOCKING THE FULL POTENTIAL OF ALGAE THROUGH CIRCULAR BIOREFINERY APPROACHES

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Algae hold significant promise for driving Europe's transition to a sustainable blue bioeconomy. Yet, current industrial processing discards up to 95% of harvested biomass, leaving valuable compounds underutilized. The CIRCALGAE project addresses this gap by developing a circular biorefinery model to convert these side streams into high-value ingredients for the food, feed, and cosmetic sectors.

The project integrates advanced green extraction technologies, including subcritical water extraction (SWE), ultrasound-assisted extraction (UAE), and high-pressure homogenization (HPH), to enhance resource efficiency and minimize environmental footprint. Three pilot-scale biorefinery schemes (TRL 7) are being implemented to demonstrate the transformation of industrial algae residues into nutrient-rich food additives, bioactive cosmetic components, and sustainable feed formulations.

CIRCALGAE's multidisciplinary consortium of universities, research centers, SMEs, and industry partners works across the entire value chain. Twelve demonstrator products are being developed, with their bioactivity validated and market potential assessed through consumer acceptance studies. By fostering circular business models, stakeholder engagement, and alignment with EU strategies such as the European Green Deal and Farm-to-Fork, CIRCALGAE supports a low-carbon, resource-efficient bioeconomy.

Launched in October 2022, the project has moved from extract characterization to prototyping for food, feed, and cosmetic applications, while actively engaging consumers to ensure market acceptance. These efforts are paving the way for a scalable, sustainable, and competitive algae-based industry in Europe, turning today's waste into tomorrow's opportunity.

# INVESTIGATION ON THE RELATIONSHIP BETWEEN REPRODUCTIVE BIOLOGY AND THE MICROBIAL COMMUNITY IN COMMON CARP (*Cyprinus carpio*)

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## Introduction

Common carp (*Cyprinus carpio*) plays an important role in fish production in Hungary. This species accounts for 82% of the annual production of fish (Bojtorné et al., 2023). Successful fish production depends on the overall stable health and favourable reproductive biology of the broodstock. The sector is highly exposed to extreme weather conditions. In addition to this factor, the sorting, transport and handling of individuals may further increase the stress on the fish, which can open the door to infections. An increasingly important area of research today is the study of microbial communities on the surface and in the organism of fish and their impact on health, growth and reproductive parameters. Following research in humans and mammals, it has been described that pathogenic and symbiotic microbes on the surface and within the body of fish influence the internal environment through complex mechanisms (Bernáth et al., 2023). When the balance is disturbed, the overall health of individuals can deteriorate, reducing their growth and thus their reproductive capacity (Amillano-Cisneros et al., 2025). The aim of our study was to investigate the relationship between the reproductive biology of carp and the effects of microbial communities.

## Materials and methods

The experimental propagation and sampling were carried out at a Hungarian fish farm (Szabolcsi Halászati Ltd.). During breeding season, 15 male and female individuals were selected from 2 wintering ponds. On the day of sampling individuals (N=10 male and female) were stripped. During sampling, the following parameters were measured and calculated: total volume of milt (TVM), volume of milt per kg of body weight (VOM), spermatozoa per millilitre (SPZ), total sperm production (TSP), total sperm production per kilogram of body weight (TNS), sperm motility and kinetic parameters, osmolality and pH of the seminal plasma and ovarian fluid. The total weight of eggs/individual (kg), the number of eggs/gram (pcs), and the pseudo-gonadosomatic index were recorded. Milt and roe samples were collected from each individual for further microbiological examination. The stripped fish were euthanized and intestinal samples were collected. A tenfold dilution series was prepared from the gut, sperm and egg samples with PBS solution. The dilution series members were spread on tryptic soy agar (TSA), de Man, Rogosa and Sharpe (MRS) agar and incubated for 96 hours at 22 °C. At the end of the incubation period, colony forming units (CFU) were counted, and morphologically distinct colonies were subcultured. The pure cultures were used for MALDI-TOF MS identification. During statistical analysis, correlation matrix and Pearson's correlation coefficient test were applied ($p<0.05$).

*(Continued on next page)*

**Results**

Negative correlation was observed between the number of CFUs of sperm TSA samples and the total sperm counts (high CFU number - low sperm count, p=0.0226). In addition, a negative correlation was identified between the number of sperm TSA CFU and the amount of semen per body weight (high CFU count, low sperm count; p = 0.0132). Negative correlation was detected between the number of semen CFU on MRSA medium and DSL (straight line distance) kinetic parameter (high CFU count but low DSL, p=0.0481). A negative correlation was identified between semen CFU on MRSA medium and the WOB (wobble coefficient) kinetic parameter (where a high number of CFU was observed, but low WOB kinetic parameter, p=0.0268). Negative correlation was observed between the formation of CFU on the egg MRSA plates and the osmolality of ovarian fluid (high germ count-low osmolality, p=0.0394).

**Discussion and conclusion**

In addition to the qualification of the gamete samples, several representatives of 17 bacterial genera were isolated and identified from the intestinal tract and gametes of the individuals with traditional culturing and molecular methods as well. The results show that several species within a genus can be found in the organs of a single individual. In the gut, members of the genera *Lactococcus* and *Lactobacillus* were abundant, whereas *Aeromonas spp*. and *Pseudomonas spp*. were more abundant in the gametes. The number of CFU was associated with negative changes in some gamete quality parameters. Several identified bacterial species are facultative pathogenic (risk group 2) organisms, therefore they may possess a biological risk on fish health.

**Acknowledgements**

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# PHYSIOLOGICAL RESPONSES OF *Artemia francsicana* TO PHBV BIOPOLYMER EXPOSURE

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**Introduction**

Currently, plastics are the most common pollutant in ocean ecosystems. Once in the environment, plastic waste breaks down into microplastics (MPs, < 5 mm) through a combination of physical, chemical, and biological processes. The presence of MPs in marine environments has profound implications for both wildlife and human health [1; 2]. In recent decades, biodegradable polymers have gained more attention due to their biodegradability and biological origin, emerging as promising alternatives to conventional plastics due to their environmental degradability and biological origin [3]. Among these, polyhydroxy-butyrate-co-valerate (PHBV), a bio-based co-polymer, that belongs to the polyhydroxyalkanoates family, has gained attention. However, its potential impact, particularly in aquatic organisms, remains largely unexplored. To address this gap, this study investigates the effects of PHBV microparticles on survival, growth, feeding rates, and ingestion in the aquatic model species *Artemia franciscana*.

**Materials and Methods**

Short-term tests were done in darkness at 25 ± 0.5 °C, and long-term tests were conducted under controlled conditions (25 ± 0.5 °C; 14:10 h light/dark). Short-term assays: ten instar II-III (**Exp 1**) and 10 juveniles (**Exp 2**) were exposed to PHBV (0 - 500 mg $L^{-1}$) in 24 and 6 well plates in triplicate, respectively. Mortality was recorded at 24 and 48h. Long-term assays: Organisms (0.5 ind $mL^{-1}$) were exposed to 25 - 500 mg $L^{-1}$ PHBV under two conditions: 1) with food (**Exp 3**): Instar II-III nauplii were exposed for 7 days with microalgae *Tetraselmis suecica.* 2) without food: Instar II-III (**Exp 4**) and juvenile (**Exp 5**) were exposed during 7 and 14 days, respectively. Two controls were included, with microalgae (CTRL (+)) and without microalgae (CTRL (-)). Survival was monitored throughout, and growth (body length) measured at start and end. Filtration and ingestion rates were assessed after 14 days at 100 mg $L^{-1}$ PHBV with food. Uptake was confirmed by exposing nauplii and juveniles to Nile Red-labeled PHBV (100 mg $L^{-1}$, 30 min) and observed under bright-field/fluorescence microscopy. Histological intestinal analysis was done after 14-day exposure (100 mg $L^{-1}$). Culture media were filtered (20 µm mesh) to collect *Artemia* fecal pellets. Filters were vacuum-dried and platinum-coated by sputtering (15 s, argon atmosphere; BalTec500). PHBV granules in fecal pellets were analyzed using scanning electron microscopy (SEM; JEOL 7001F, 5 kV). Negative controls included commercial polymers unexposed to *A franciscana*.

**Results**

Short-term exposure showed <20% mortality of both nauplii and juveniles at all concentrations (**Exp 1&2**). In long-term tests, only 500 mg $L^{-1}$ caused significant mortality in nauplii (76% by day 2); other concentrations had >85% survival with food (**Exp 3**). Without food (**Exp 4**), nauplii survival declined with concentration. CTRL (−) had 100% mortality by day 5. CTRL (+) maintained 93% survival. Juveniles (**Exp 5**) showed highest survival at 250 mg $L^{-1}$ (90%) by day 7 and dropped to 17% by day 14. CTRL (+) had 83% survival. Growth was unaffected with food (**Exp 3**); and significantly reduced without food (**Exp 4**) compared to CTRL (+), but not between treatments. In contrast, no growth differences were observed in juveniles. Feeding behavior were not affected. PHBV ingestion was confirmed in both stages, with particles visible in the digestive tract and fecal pellets (**Fig 1**). No major histological damage was observed, but intestinal epithelium appeared disorganized with reduced vacuolation and areas fragmented (**Fig 2**). Granules present in *Artemia* feces showed increased surface roughness and structural deformation relative to control particles (**Fig 3**), supporting the hypothesis that *A. franciscana* can process or partially

*(Continued on next page)*




Figure 1: Images of PHBV fluorescence stained with Nile red in the digestive tract of *A. franciscana* nauplii (A - D) and 14 days organisms (E - H). Images of fecal pellets (I - L).

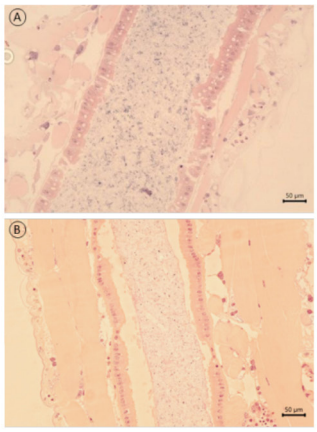


Figure 2: Instestinal tissue of *A. franciscana*. A) control B) PHBV 100 mg $L^{-1}$

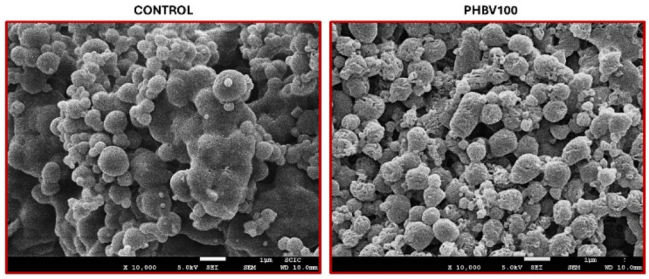


Figure 3. Scanning electron microscopy (SEM) images of PHBV granules before ingestion (left) and after ingestion, as recovered from the feces of *A. franciscana* (right).

assimilate PHBV through digestive activity. The results showed that *A. franciscana* ingests PHBV, which supports survival in the absence of food, especially in juveniles, but does not promote growth. PHBV is less harmful short-term exposures than some conventional plastics, however in long-term exposure under food-limited conditions require further study. These findings contribute to the environmental risk assessments of biodegradable plastics in marine systems.

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# DAILY GENE EXPRESSION PATTERNS IN THE DIGESTIVE GLAND OF JUVENILE *Octopus vulgaris* IN THE WILD

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## Introduction

Biological rhythms are intrinsic timekeeping mechanisms that regulate physiological processes in living organisms, including daily feeding patterns. Feeding behaviour is a key determinant of an organism's nutritional status, and the timing of food intake plays a critical role in overall health and welfare (Asher and Sassone-Corsi, 2015). Although subadult and adult *Octopus vulgaris* are known to be mostly nocturnal predators, nothing is known about the activity rhythms of juveniles in the wild, active predators mostly on amphipods (Escolar et al. 2024). Understanding the molecular rhythms of its digestive system is essential for developing optimized feeding strategies that promote growth, enhance physiological efficiency, and ensure animal welfare under culture conditions. In this study, we investigate the gene expression patterns in the digestive gland (DG) of recently settled, juvenile *O. vulgaris* over a 24-hour cycle using RNA-sequencing analysis.

## Materials and Methods

***Collection of material:*** *O. vulgaris* individuals were collected on November 11, 2023, using experimental juvenile octopus' collectors (Escolar et al. 2021) placed at 5–15 m depth on sandy bottoms off Vilanova i la Geltrú (NW Mediterranean). A total of 35 individuals were collected between 06:30 and 11:00 h. Specimens (mean weight: 427 ± 622 mg; range: 183–3428 mg) were kept in small containers onboard, transported to aquarium facilities of the ICM-CSIC located at 50 km and provided by an open seawater system, and placed in rearing tanks by 13:00 h. They were housed in six 5 L polyethylene tanks (8 individuals/tank) with refuges, under natural photoperiod and ambient seawater temperature of 17.2 °C. Light intensity at tank bottom was 15 lux. Natural sunrise and sunset during the experiment were at 07:45 and 17:45 local time, respectively. No food was provided.

***Sampling for endogenous rhythms analysis:*** Five individuals were sampled every 4 hours across seven time points starting at 12:00 h (t0 (midday)), 16:00 h, 20:00 h (t4, t8 (afternoon, evening)), 24:00 h (t12, (midnight)), 04:00 h, 08:00 h (t16, t20, (early morning, sunrise)) and 12:00 h (t24 (midday, after 24h)). Digestive glands (DG) were dissected after the death of the individuals, preserved in RNAlater at 4ºC during 24h, and stored at -80 °C for transcriptomic analysis. Animal welfare procedures followed EU guidelines (Directive 2010/63/EU) and cephalopod-specific recommendations (Fiorito et al., 2015).

***RNA extraction and sequencing:*** Total RNA from pooled DG samples of each time point was extracted using the RNeasy Mini Kit (Qiagen). RNA quality was assessed via NanoDrop 2000. Library preparation and sequencing were performed by Biomarker Technologies (BMK GmbH, Germany) using Illumina NovaSeq X (PE150).

***Bioinformatics and statistical analysis:*** Raw FASTQ reads were cleaned using custom Perl scripts. Quality metrics (Q20, Q30, GC content, duplication rates) were calculated. Clean reads were aligned to the *O. vulgaris* genome (NCBI Accession number: GCA_951406725.2) using HISAT2 v2.2.1 (Kim et al., 2019). Transcript reconstruction and quantification were done with StringTie (v2.2.1; Kokava et al., 2019, FPKM method), and transcriptome quality assessed via GffCompare (v0.12.6; Pertea & Pertea, 2020). Time-course expression data were normalized using edgeR (TMM method, Robinson and Oshlack, 2010), filtering low-expression genes (<0.5 CPM). Differentially expressed genes (DEGs) were identified ($p < 0.05$, $FC \geq 2$). Mfuzz

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(Kumar and Futschik, 2007) and TMixClust (Martínez-Bueno and Díaz, 2021) were used for soft clustering and time-series visualization, respectively; Heatmap3 for clustered heatmaps (Zhao et al., 2014). DESeq2 was used for pairwise DEG analysis (Love et al., 2014). GO enrichment was performed using topGO (Alexa & Rahnfürer, 2010) and GOseq (Young et al., 2010) adjusting for gene length bias). Pathway analysis was conducted using KEGG (Kanehisa et al., 2008).

**Results**

Time-course analysis of differentially expressed genes in the digestive gland of *O. vulgaris* juveniles identified 3 different clusters with distinct temporal patterns. Clusters 1 and 2 show opposing expression profiles, suggesting opposed regulation between gene sets, while Cluster 3 displays an intermediate pattern. Cluster 1 shows two peaks of maximum expression: one at midday (t0 and t24; 12:00 h) and another early morning at 4:00 h (t16). In Cluster 2, the peaks occur from afternoon to midnight (t4–t12; 16:00–00:00 h) and at sunrise (t20; 8:00 h). Cluster 3 reaches its maximum expression at evening (t8; 20:00 h) and early morning (t16; 04:00 h). Enriched GO terms observed include: metal ion binding (associated with enzymes and metabolic processes), ATP binding and nucleic acid binding (involved in nuclear and enzymatic functions), integral component of membrane (related to transporter proteins or receptors), and response to stimulus (adaptation to external signals, such as the light/dark cycle).

The KEGG pathway enrichment analysis of differentially expressed genes (DEGs) between 8:00 h (t20) and midday (t24 and t0), peak at 12:00 h, suggest the completion of digestive activity, metabolic resetting, and preparation for the next phase. Between midday (12:00 h, t0) and afternoon (16:00 h, t4), with a peak at 16:00 h, enriched pathways suggest metabolic shifts**,** likely reflecting active digestion and nutrient assimilation. In the transition from afternoon (16:00 h, t4) to evening (20:00 h, t8), with a peak at 20:00 h, enriched pathways likely represent the post-absorptive phase, during which nutrients are processed and stored. The organism also appears to prepare for nocturnal activity, consistent with the predatory behaviour typically observed in *O. vulgaris* (Rosa et al., 2024).In summary, the temporal variations observed over a 24-hour cycle, together with the KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in the digestive gland of octopuses, reveal complex rhythmic patterns in digestive system activity. These changes are closely aligned with the light/dark cycle and likely reflect underlying circadian metabolic processes.

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# LONG-TERM PERSISTENCE AND PUTATIVE VERTICAL TRANSMISSION OF RGNNV/SJNNV BETANODAVIRUS IN GILTHEAD SEA BREAM (*Sparus aurata*)

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**Introduction**
The gilthead sea bream (*Sparus aurata*) was historically considered resistant to viral nervous necrosis (VNN), an important disease caused by Betanodavirus. However, recent reports have identified significant larval mortality events associated with a reassortant strain of nervous necrosis virus (NNV), the RGNNV/SJNNV, composed of an RNA1 segment from the RGNNV genotype and an RNA2 segment from the SJNNV. Although some marine species have been confirmed to transmit the NNV vertically, this transmission route remains unverified in *S. aurata*. Moreover, the dynamics of long-term viral persistence in this species are still poorly understood.

**Materials and methods**
Previous experimental infections were conducted via immersion on sea bream larvae at 35 and 75 days post-hatch (dph) using the RGNNV/SJNNV strain. Following these initial trials, the surviving fish were maintained under controlled conditions and monitored for up to 7 years to assess long-term viral persistence in brain, retina and gonadal tissues by molecular and virological methods. Reproductively mature, persistently infected individuals were induced to spawn, and samples of eggs, sperm and resulting larvae were analyzed for the presence of Betanodavirus to evaluate the potential for vertical transmission.

**Result**
Previous results from larvae infection showed the presence of Betanodavirus in all larvae starting from 1 day post-infection, with peak viral loads at 10 dpi and comparable replication profiles across both age groups. Histological and immunohistochemical analyses confirmed early viral localization in the brain and retina. No clinical signs nor mortality were observed.

Asymptomatic fish remained positive for Betanodavirus up to 7 years post-infection, with persistent virus detected in brain, eye, and gonadal tissues by RT-qPCR. However, viral distribution within the gonads was not homogeneous: the presence of viral RNA in ovaries and testis showed intra-organ variability. Similarly, viral RNA was detected in both sperm and eggs, but not consistently across all stripping events from the same positive-testing breeder. Furthermore, within a single spawning event, the presence of the virus was not evenly distributed among gametes. In our experimental trials, all newborn larvae tested negative for Betanodavirus by RT-qPCR up to 28 days post-hatch.

**Conclusions**
This study demonstrates that *S. aurata* can harbor long-term persistent RGNNV/SJNNV Betanodavirus infections in both neural and reproductive tissues, without any clinical manifestations. The detection of viral RNA in gametes, along with uneven distribution patterns, suggests a potential—though not yet confirmed—route for vertical transmission. These findings highlight the critical role of broodstock in the maintenance and possible spread of Betanodavirus in aquaculture settings, and stress the importance of implementing sensitive diagnostic protocols and rigorous screening strategies for broodstock management in marine hatcheries.

The research leading to these results has received funding by the Italian Ministry of Health project RC IZSVE 09/15 and RC IZSVE 13/22

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# SPAWNING BEHAVIOUR OF TWO COMMERCALLY IMPORTANT NESTING PERCIFORMES

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## Introduction

The pikeperch *(Sander lucioperca)* is a commercially important species in Europe, with well-established protocols of artificial reproduction. However, these protocols are labor-intensive and often require repeated handling of broodstock females, which can induce acute stress and increase broodstock mortality (Rónyai, 2007,) what can even potentially negatively affect offspring quality (Rasal et al., 2024). The largemouth bass (*Micropterus salmoides*) is primarily significant in Europe for recreational angling. However, its low-fat flesh and high-quality fillet have made it globally popular among health-conscious consumers (Parmar et al., 2022). At the same time, rising expectations regarding product quality have led to a decline in demand for common carp (*Cyprinus carpio*) (Hu et al., 2023), resulting in China's annual production of largemouth bass for human consumption approaching one million tons (Liu et al., 2022). However, most of the production is seasonal and/or based on nest spawning technology, which may involve limited controllability and low biological diversity. Despite the high production volume, no well-developed artificial reproduction protocol has been published to date, while the precise spawning ethology of the species remains undescribed. Given these challenges, the objective of the present study was to describe the spawning indicators in both species through camera observation and recording.

## Materials and methods

In January 2025, during preseason pikeperch reproduction, six pairs of breeders were hormonally stimulated with salmon gonadoliberin analog using the warming thermal regime (Ljubobratović et al., 2021). The 6 pairs of breeders were placed in two 4m$^3$ tanks, divided into three spawning chambers, creating a total of six breeding units. In each unit, an artificial nest was placed, made of artificial grass, and an underwater lighting system, programmed to a 12:12 LD photoperiod, with a camera above each compartment. After spawning, nests containing fertilized eggs were placed individually into conical incubators for incubation, and the hatched larvae were subsequently counted. In April 2025, six pairs of largemouth bass breeders were placed into three spawning tanks, each tank with two pairs, with three different setups: D - divided tank: the tank separated in the middle by a partition net, one nest per pair of breeder; CH - chambered nest tank: first and last third the tank each with a nest at in the cover with an empty third in the middle and securing free movement throughout the tank and hiding are for females; F – free tank: one nest at each end of the tank, securing free movement throughout the tank without any chambers included. Fish were hormonally stimulated with human chorionic gonadotropin three days upon stocking, and the behavior of the fish was further camera-monitored. After spawning, eggs were collected from the nests, their number and embryo survival were evaluated, and stocked in Zug jars for incubation.

## Results

During the pike-perch spawning experiment, spawning was observed in all six pairs on the 8th and 9th days after hormonal stimulation. After placing the artificial nests into the incubators, the number of hatched larvae was assessed and ranged from 15.200 to 28.899.. In the case of the largemouth bass experiment, among three setups, the tank equipped with half partitions proved to be the most effective. Two nests contained eggs, and CH treatment yielded ~80 thousand live eggs while the F nest contained close to 40 thousand live eggs. The hatching rate was 100% for both nests. Both at the beginning and the end of the experiment, the males were spermiating.

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**Discussion**

Artificial intelligence is increasingly gaining ground in various areas of aquaculture and has already been successfully applied in several production processes, such as feeding management and health monitoring (Mandal & Ghosh, 2024). Animal welfare is also receiving growing attention, particularly in relation to behavior-based indicators, which have recently come into focus (Barreto et al., 2022). By utilizing spawning indicators based on video monitoring, new protocols can be developed that not only improve the survival rate of broodstock but may also positively affect the quality of eggs and larvae. Previous research has shown that problems affecting the parents can have molecular-level effects on their offspring, influencing their later performance (Rasal et al., 2024). The present research led to the description of the ovulation indicators that will be further utilized to set the new spawning protocol, reducing the fish handling and labor requirements.

**Acknowledgements**

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# FROM COZY INDOORS TO ROUGH SEAS – GROWTH OF LARGE RAS RAISED RAINBOW TROUTS TRANSFERRED TO SEA CAGES IN DIFFERENT SEASONS

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## Introduction

Due to new operation models (e.g. offshore farming) demand for larger fish for sea grow-out is increasing (Bergheim et al., 2009, Ytrestøyl et al., 2022). RAS offers possible way of producing larger fish with control on nutrient output and stable environmental conditions for fish. Sea cage grow-out producers in seasonal environment would preferably have all the fish at the same time in spring after the ice cover has left, while RAS is most cost-effective when it does not have to produce fish with all-in-all-out -fashion. However, it is uncertain how the fish grown to larger sizes in stable RAS conditions will adapt to seasonal sea grow-out environment (Lai et al. 2024, Ytrestøyl et al., 2022). In this study we tested how RAS grown 500 g rainbow trout perform in sea cages when they arrive to sea in different times of the year.

## Materials and methods

At early summer of first grow-out period < 50 g fish from early hatched egg batches were transported from both freshwater flow-through (Control) and RAS (RAS1) farm to sea cages.

Fish from RAS1-group were also kept in RAS and grown to size > 500 g after which they transferred to sea cages at late autumn of first grow-out period (RAS2). Fish from late hatch egg batch were grown in RAS until size > 500 g, and then transported to sea cages in late winter/early spring of second grow-out period (RAS3). From RAS3-group 5 g fish were also taken to colder flow-through water for the summer of first grow-out period. These fish were returned to RAS at size of approx. 50 g at autumn and subsequently raised in RAS until > 500 g when they were transferred to sea cage at late spring at the onset of the second grow-out period (RAS4).

All the fish groups were grown with automated feeders according to feed manufacturer's table adjusted to correspond optimal feeding by visual inspection of appetite (Optimised). In addition, Control and RAS4 -groups were fed with feeders that are adapt (Adaptive) to the feeding amount based on fish appetite.

## Results

Control and RAS1 -groups had a good growth in optimised feeding. However, fish transferred as > 500g from RAS showed difficulties to adapt for the grow-out in sea cages (Fig.1)

## Discussion

Dramatic reluctance of RAS-raised larger rainbow trouts to feed and grow in sea cages makes combination of RAS and sea cage farming challenging, and more causal and physiological investigations to solve this puzzle are needed (Hänninen et al. in prep.).

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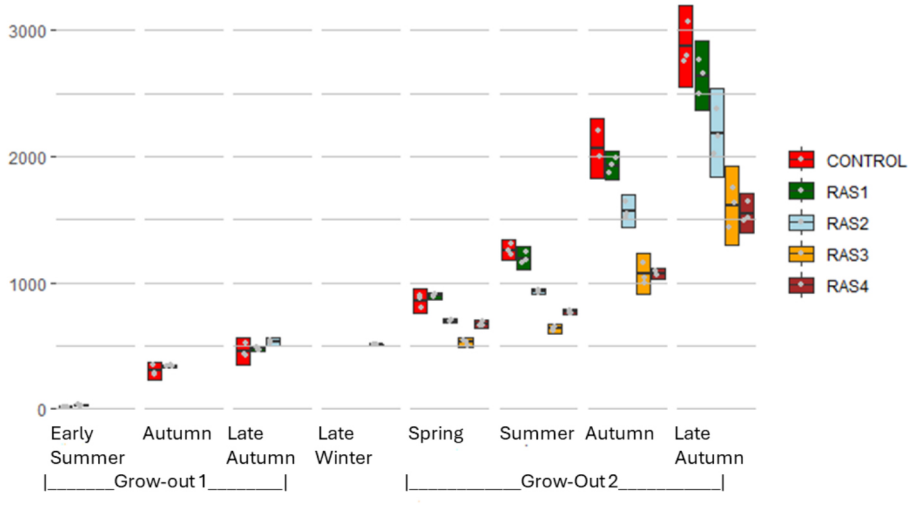


Figure 1. Fish growth in optimized feeding. Mean weight (horizontal line), 95 % confidence intervals (box), individual means for replicate cages (grey dots within box) throughout the both grow-out periods.

Table 1. Fish growth in adaptive feeding.

| Group | Control | | RAS4 | |
|---|---|---|---|---|
| Grow-out 2 | Mean weight (g) | Mean FCR | Mean weight (g) | Mean FCR |
| Spring | 868 | - | 678 | - |
| Summer | 1494 | 1.15 | 820 | 1.48 |
| Autumn | 2564 | 1.51 | 1275 | 1.82 |
| Late Autumn | 3875 | 1.09 | 2065 | 1.43 |

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# RISE LIKE A PHOENIX - START-UP OF BRACKISH WATER RAS SYSTEM UNUSED FOR NEARLY TWENTY YEARS

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**Introduction**
The start-up and activation of RAS systems is complex technological and biological research subject (Pulkkinen et al. 2018). Parainen brackish water RAS comprises of two separate systems. The RAS has been working as all-in-all-out -principle for two decades, where the system is emptied from water for the summer every year, and activated again in autumn. In addition, due to logistic and technical reasons for almost two decades only one of the systems has been used in experiments, and the other system's biofilters have been dry. As a part of joining Finnish Marine Research Infrastructure (https://www.finmari-infrastructure.fi/) Parainen RAS was upgraded to maintain temperature separately in two systems. This also provided us with an unique opportunity to test how fast will the biofilter with extended period of unuse start to work. Study questions: 1) how fast will the biofilters in two systems start to work, 2) how comparable results of fish trials are in two different systems, 3) how much fish we can we can raise within the system.

**Materials and methods**
The RAS systems were started similarly with chemicals before fish arrived to facility. The temperature of the systems were set to 14,5 C. The fish arrived 9.1.2025 (mean weight 568 g, n=180) and were divided to two systems. Fish were grown with automated feeders according to feed manufacturer's table adjusted to correspond optimal feeding by visual inspection of appetite. The fish growth and water quality parameters were monitored through the experiments. The fish were first grown in two separate systems, and at the end all the fish were transferred to system 1 to see whether we can reach the upper limit of the system capacity.

**Results**
Comparison of the two systems.

**Discussion**
The start-up of System 2 after nearly two decades of dry biofilters was astonishingly fast, after initial peak of nitrite, the water quality parameters came to same level with System 1, and the fish growth was similar in both systems Upper limits for System 1 could not be reached within experimental period, but it is evident that the system is capable to function as full RAS (<20 % of water renewal, 800 l / kg feed, > 70 kg /$m^3$). Besides interesting experimental system for fish trials, the biofilters themselves provide subject of further studies.

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Table 1. Fish growth parameters (means) in two separate systems.

| | System1 | | | | System 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Weight | FCR | SGR | TGC | Weight | FCR | SGR | TGC |
| 9.1.2025 | 575 | - | - | - | 565 | - | - | - |
| 5.2.2025 | 943 | 0.88 | 2.06 | 4.28 | 887 | 0.87 | 1.88 | 3.85 |
| 5.3.2025 | 1408 | 0.95 | 1.66 | 3.99 | 1342 | 0.95 | 1.59 | 3.78 |
| 26.3.2025 | 1806 | 1.03 | 1.31 | 3.52 | 1722 | 1.01 | 1.31 | 3.46 |

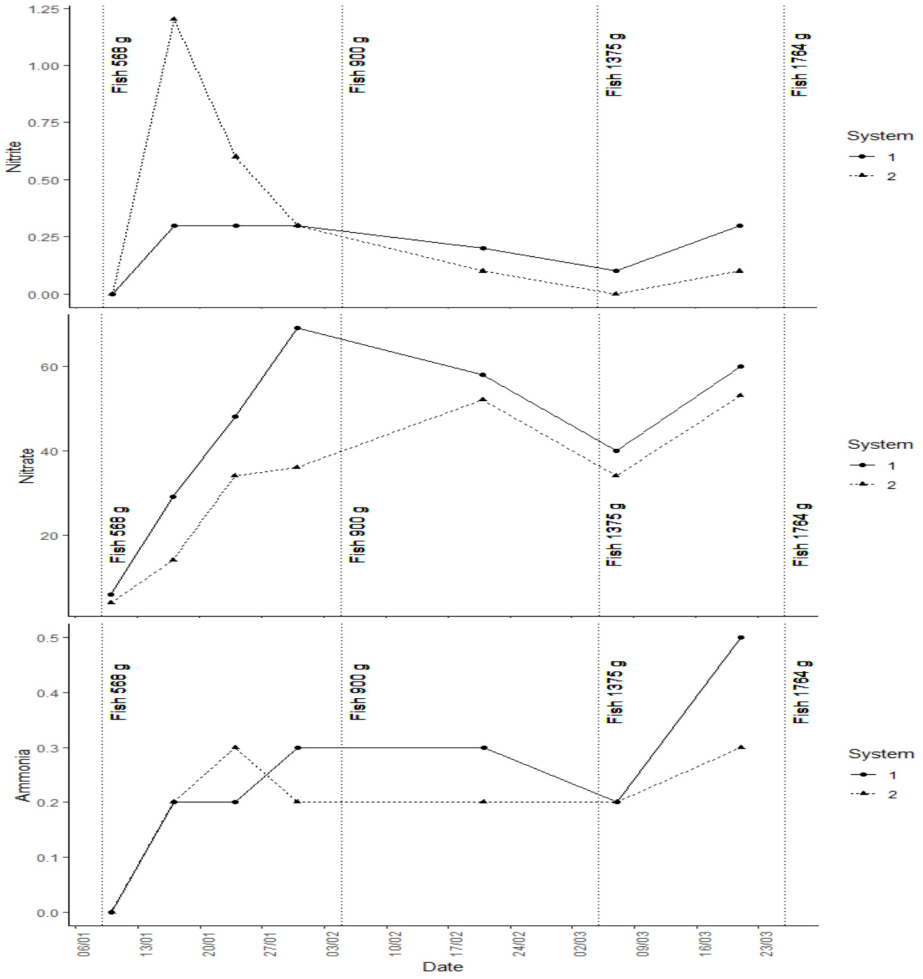


Figure1. Water quality parameters in two systems..

Trying to reach the upper limits

Table 2. Fish growth and water quality parameters [mean (min - max)] in System 1.

| | Weight (kg) | FCR | SGR | TGC | Nitrite | Nitrate | Ammonia |
|---|---|---|---|---|---|---|---|
| 26.3. | 1.8 | - | - | - | - | - | - |
| | 1.7-1.9 | - | - | | - | - | |
| 24.4. | 2.3 | 1.3 | 0.9 | 2.7 | 0.53 | 92 | 4.4 |
| | 2.3-2.3 | 1.1-1.8 | 0.8-1.1 | 2.4-3.0 | 0.3-0.8 | 42-120 | 0.8-6.2 |

# EXPANDING THE AQUACULTURE RESEARCH TOOLBOX: VALIDATING CANDIDATE GENES IN SAB-1 AND DLB-1 CELLS VIA OPTIMIZED DELIVERY OF INTERFERENCE RNA AND CRISPR/CAS9

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## Introduction

Aquaculture is the fastest-growing food production sector worldwide. Despite this positive trend, the industry faces several challenges that keep it from reaching its full potential. A major limitation is the lack of understanding of the molecular pathways involved in regulating reproduction, growth, and disease resistance of the farmed species.

In this context, fish cell lines are gaining relevance as valuable models for exploring these complex traits. Especially in combination with molecular tools such as RNA interference and CRISPR/Cas9 genome editing, which can dissect the genetic basis of traits of interest using the appropriate cellular models for aquaculture species. However, the application of these technologies remains limited due to technical constraints.

Here, we present optimized methods for the delivery of RNA and CRISPR/Cas9 ribonucleoproteins (RNPs) into cell lines derived from two major aquaculture species: *Sparus aurata* and *Dicentrarchus labrax*.

As proof of concept, we used the CRISPR/Cas9 system to edit genes located in a major quantitative trait locus (QTL) associated with resistance to Viral Nervous Necrosis (VNN) in *D. labrax* (Mukiibi et al., 2025). VNN is caused by the Nervous Necrosis Virus (NNV) and affects the survival and growth of multiple species, including both *D. labrax* and *S. aurata*. Despite its impact on aquaculture production, no prevention or treatment is currently available for this disease. Using our optimized protocol, we edited the previously mentioned QTL in cell lines from *S. aurata* and *D. labrax* (Arana et al., 2025), which will be used to investigate the genetic basis of the resistance phenotype, thus making a first step towards the generation of VNN-resistant fish.

## Materials and methods

We selected two cell lines from the species of interest: SaB-1 from *S. aurata* and DLB-1 from *D. labrax*. In these models, we optimized delivery conditions with two different approaches: electroporation and chemical transfection. To this end, we transfected a tracrRNA-ATTO™ 488 and evaluated transfection efficiency 48 hours later with flow cytometry (CytoFLEX flow cytometer, Beckman Coulter Life Sciences). We also assessed cell viability after transfection using an MTT assay.

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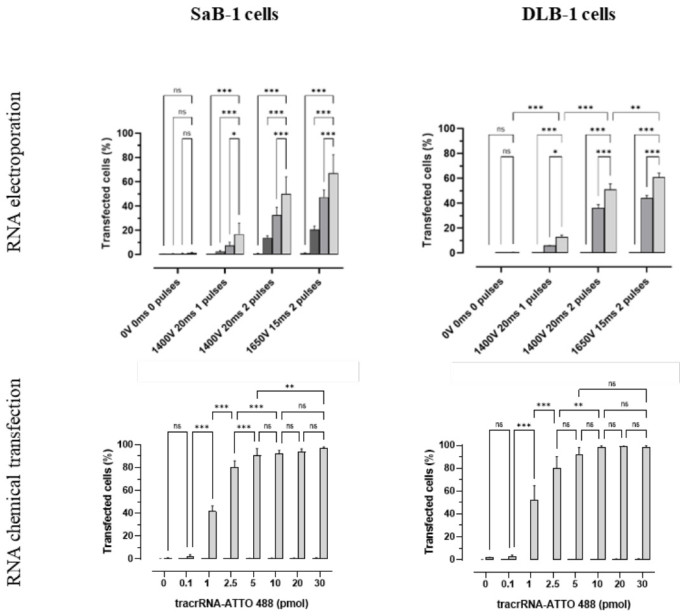


Figure 1. Transfection efficiencies in SaB-1 and DLB-1 cells using electroporation and chemical transfection with a tracrRNA-ATTO 488.

## Results

We optimized RNA delivery using a fluorescent tracer and achieved good transfection efficiencies in both cell lines with electroporation and chemical transfection (Figure 1). Subsequently, we applied the optimized electroporation parameters to edit a QTL associated with VNN resistance using the CRISPR/Cas9 system. The results revealed major differences between the two cell lines: while over 90% of SaB-1 cells were successfully edited, only 30% of DLB-1 cells showed signs of genome modification.

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# OPTIMIZING PLANT-BASED DIETS FOR RAINBOW TROUT: GROWTH AND PLASMA METABOLITE EFFECTS OF CEAMSEA SUPPLEMENTATION

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## Introduction

The aquaculture sector plays a key role in meeting the increasing global demand for seafood, experiencing substantial growth and making a significant economic contribution worldwide (1). In Europe alone, aquaculture production has reached record levels in recent years, emphasising the need for sustainable growth strategies. A critical challenge for the industry is developing sustainable, nutritionally adequate aquafeeds, given the environmental, economic and resource constraints that are increasingly limiting the use of traditional marine-derived ingredients such as fishmeal (2). The shift towards more sustainable aquafeeds has led to an increased reliance on plant-based ingredients in salmonid diets. However, replacing marine-derived components entirely can negatively impact the growth and metabolic balance of carnivorous species such as rainbow trout (*Oncorhynchus mykiss*). In this context, functional ingredients are being explored to improve the nutritional value of plant-based formulations (3). Among these, CEAMSEA, a marine algae-derived ingredient currently used in the agri-food industry for its functional properties, emerges as a promising additive for aquafeeds. Including it in plant-based diets may help to counteract the nutritional limitations commonly associated with high levels of plant proteins in carnivorous fish. Therefore, we hypothesise that supplementing a plant-based diet of rainbow trout with 2.5% or 5% CEAMSEA will improve growth performance, physiological indices and plasma metabolite profiles, thereby enhancing the overall health and growth efficiency of fish fed a plant-based diet.

## Materials and methods

Four experimental diets were formulated to evaluate the impact of CEAMSEA supplementation on rainbow trout. A fishmeal-based diet (CTRL) was used as the positive control diet, while a plant-based diet (V0) served as the negative control. Two additional diets were derived from V0 by supplementing it with 2.5% or 5% CEAMSEA obtained from CEAMSA (Porriño, Spain), resulting in four isoproteic, isoenergetic and isolipidic formulations: CTRL, V0, V2, and V5. Diets were randomly assigned to triplicate groups of 30 rainbow trout juveniles (initial weight 18.3 ± 3 g), which were distributed into twelve tanks of an open rearing system in 100 L-fiberglass within an open rearing system at INRAE's experimental facilities in Donzacq (France). Fish were exposed to natural photoperiod conditions and a constant water temperature of 17 ± 2 ºC; and fed their respective diets to apparent satiation once daily. After 84 days, all fish were individually weighed and measured. Hepatosomatic (HSI) and viscerosomatic (VSI) indices were calculated, and blood samples were collected for plasma metabolite analysis, using 15 fish per treatment (5 fish per tank). Growth performance, feed efficiency and physiological parameters were then compared across the different dietary treatments to evaluate the effect of including CEAMSEA in plant-based diets. Statistical analysis was performed using GraphPad software. A one-way ANOVA was conducted and, when significant effects were detected ($p < 0.05$), a Tukey's post hoc test was performed.

## Results and discussion

All experimental diets promoted substantial growth, with fish increasing their initial body weight by five to seven times over the 84-day feeding trial, indicating good overall acceptance. As expected, the control diet containing 25% fishmeal (CTRL) produced the best growth results, with the highest final body weight (128.2 g ± 3 g) and weight gain (110 g ± 6 g). Growth was significantly impaired by the plant-based diet without supplementation (V0), resulting in a 24% lower final weight (97 g ± 14 g), and a significantly reduced weight gain compared to the CTRL group. However, the inclusion of CEAMSEA at 2.5% (V2) and 5% (V5) effectively restored growth performance to levels comparable to CTRL, eliminating the significant differences observed with V0. Furthermore, although V5 was superior to V2 in both final weight and weight gain, apparently in a dose-dependent manner, no statistically significant differences were detected between the two supplemented groups.

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Interestingly, the condition factor (K) was significantly higher in the V5 group (1.35) than in the CTRL group (1.12), with no significant differences observed among the other groups. Despite having slightly lower final weights, fish fed the V5 diet exhibited better body conformation, suggesting that supplementing with 5% CEAMSEA may enhance somatic growth or promote more compact body development in rainbow trout under plant-based feeding conditions. The specific growth rate (SGR) followed a similar trend, with the CTRL group showing the highest value (2.32), which was significantly higher than those of the V0 (1.99) and V2 (2.05) groups. However, no significant difference was found between the CTRL group and the V5 group (2.13), which reinforces the potential of 5% CEAMSEA inclusion to restore growth performance in plant-based diets. In terms of physiological indices, no significant differences were observed in VSI between the groups. However, HSI was significantly lower in fish fed the V2 diet than in those on the CTRL group. Further metabolic analysis is required to clarify the underlying mechanisms and implications.

In terms of plasmatic metabolites, no significant differences were observed in glucose, lactate or triglyceride levels between groups. Interestingly, vegetable-based diets (V0, V2 and V5) exhibited higher levels of non-esterifies fatty acids in plasma compared to CTRL group.

Overall, these findings suggest that growth performance and body condition of rainbow trout were improved by supplementing a plant-based diets with CEAMSEA at 2.5% and 5%. The 5% inclusion level was particularly effective in partially bridging the performance gap with a fishmeal-based diet. These results demonstrate CEAMSEA's potential to improve the nutritional quality of sustainable aquafeeds. Further research is needed to explore the effects of higher inclusion levels and to elucidate the underlying metabolic and histological mechanisms.

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**Acknowledgements**

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# AMINOACID COMPOSITION OF MICROBIAL PROTEIN EXTRACT OF RUMINAL ORIGIN TO SALMON FEEDING

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## Introduction

The need to seek new high-quality protein alternatives to feed salmon has motivated the emergence of innovative alternatives for this purpose. Fish depend externally on essential amino acids to meet their requirements, so if these are not provided in the diet in adequate amounts, fish may suffer detrimental consequences in their physiology, metabolism, and overall health (Jones et al., 2020). On the other hand, microbial proteins of ruminal origin have a high biological value, highlighting their high content of essential amino acids, making them a potential alternative to generate a protein extract for nutritional purposes (Johansen et al., 2018). To obtain these protein extracts from the ruminal microbiota, it will be necessary to cultivate the inocula in bioreactors, which means that the technique used to generate these extracts could affect the amino acid composition. Consequently, the objective of this research was to evaluate the amino acid composition of two protein extracts of microbial origin obtained through two protein purification techniques.

## Materials and methods

The ruminal fluid (RF) was collected from six Suffolk Down sheep using the oro-ruminal technique. The donor sheep were fed with timothy hay under maintenance requirements. The ruminal protein extracts were obtained under two purification alternatives: 1. Centrifugation of the RF at 4000 rpm at 4°C, generating a pellet rich in microorganisms; subsequently, this pellet was freeze-dried and stored under refrigeration until its amino acid analysis. This extract was designated Raw Extract of Rumen Microorganisms (EBMR). 2. After obtaining the pellet of microorganisms, it was resuspended in potassium phosphate buffer pH 7.0 ($KH_2 PO_4$), and then subjected to disruption by sonication (ultrasound), followed by centrifugation at 12,500 rpm at 4°C. To isolate the proteins, they were precipitated with ammonium sulfate (550 g/l) using the *salting out* technique. Then, a second type of protein extract was obtained, termed Pure Extract of Microbial Proteins (EPPM). After generating the two types of extracts, the crude protein (Kjeldahl) and its amino acid profile were determined through HPLC (high-performance liquid chromatography). The experimental design was completely randomized with a factor with two levels (type of extract). Three replicates per treatment were performed. To evaluate the differences in means, the Mann-Whitney Non-Parametric Ordinal Test was used. It was inferred that the differences were significant when $P<0.01$. The statistical analysis was conducted using the IBM SPSS Statistics 19 software.

## Results

The Crude Protein (CP) content was 48.28% and 66.61% for the EBMR and EPPM treatments, respectively, clearly evidencing the difference in the level of protein purification that implies generating cell disruption and subsequently precipitating the released cellular proteins with ammonium sulfate. Regarding the amino acid analysis, a high biological value of the analyzed proteins was observed, along with differences between treatments in most of the compared amino acids (Table 1). The values found, both in CP content and amino acid quality, position these types of extracts as an alternative to be considered as supplements in salmon feeding. The Relationship of Essential Amino Acids (EA)/Total Amino Acids (AA) for each type of extract generated was: EBMR: EA/AA=38.7% and for EPPM: EA/AA=51.2%.

## Discussion and conclusión

Nutritional differences were observed between the EBMR and EPPM treatments, mainly in protein content and its amino acid composition. The observed CP content for EBMR and EPPM extract, positions it favorably to be used as an ingredient in formulation for salmon feed, considering that soybean meal contains 45% and fish meal around 60% CP. An adequate biological value was found in both protein extracts for the feeding of salmon, with a higher content of essential amino acids,

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especially the extract EPPM. Regarding the lysine content, a 4.07% was observed for the EBMR extract and 4.83% for EPPM. These values are promising; between a 4.8 y 8.4% has been reported for soybean meal (Anyiam et al., 2025) and an 5.16% for fish meal (Zhang, 2024). In the case of methionine, this value was 3.61% for EPPM, compared whit 1 and 1,3% to soybean, reported by Anyiam et al. (2025), and 1.77% for fish meal (Zhang, 2024). On the other hand, the detected difference between the types of extracts may have been due to the fact that different proteins were released at different concentrations (intracellular origin), resulting in amino acidically different extracts. The foundation of this phenomenon is based in the fact that sonication (cell rupture) may have released different proteins, or peptides, that were trapped in more refractory cell compartments, or proteins located in the bacterial walls strongly bound to nitrogen compounds or other structural molecules of these walls. Apparently, the EPPM extract, through its purification technique, managed to concentrate proteins of better biological quality. For any of the extracts, the amino acid profile found positions these types of microbial-origin extracts favorably as potential alternatives to be used as additives in the formulation for salmon feed.

**Acknowledgement**
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# POOR PERFORMANCE OF VEGAN DIETS FOR GOLDEN GREY MULLET (*Chelon auratus*) FEEDING COMPARED WITH AN EXTRUDED FEED FOR CARNIVOROUS FISH

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## Introduction

Current aquaculture focuses on carnivorous species that require large amounts of protein and lipids derived from fish meal and oils, as well as by-products from the meat industry. The introduction of omnivorous species in Mediterranean aquaculture makes it possible to use exclusively plant-based raw materials for their production (Gisbert et al. 2016). This research explores the use of different raw materials for the whole replacement of animal-based ingredients in the diet of golden grey mullet (*Chelon auratus*).

## Materials and Methods

A total of 180 fish weighing between 3 and 9 grams (5.27±1.32 g) were used. They were distributed into 12 tanks of 216 liters (15 fish per tank) and kept in a semi-closed seawater system with temperature controlled (25.5±0.69°C). Four diets were tested by triplicate: one commercial gilthead seabream diet as control, and three vegan diets based on a mixture of cereals, legumes, vegetables and seeds. The vegan diet contained 20% rice, 20% wheat semolina, 20% lentils, 10% quinoa, and 30% broccoli (Vegan). In the seed diets, 5% of the rice was replaced by sunflower seeds in one diet (VeganGi) and chia seeds in the other (VeganCH). The ingredients were cooked, except for the seeds, which were previously ground and sieved to a particle size of 1–2 mm, suitable for the fish. The fish were fed to satiety three times a day until day 40, when they were weighed to calculate growth and feed utilization indices: initial weight (Wi), final weight (Wf), mean weight (Wm), weight gain (WG), absolute feeding rate (AFR), specific feeding rate (SFR), both also calculated on a dry weight basis (AFRd and SFRd), absolute growth rate (AGR) and specific growth rate (SGR), as well as feed efficiency ratio (FE) and feed conversion ratio (FCR), with their respective dry weight values (FEd and FCRd).

## Results

The composition of the plant-based diets differed greatly in macronutrients compared with the commercial diet, with the latter containing higher protein and lipid content and much lower carbohydrate content (Table 1, $P < 0.05$).

Fish fed commercial diet achieved significantly higher growth and feed utilization indices than those fed plant-based diets ($P<0.05$), despite feed intake being much higher in the plant-based diets ($P < 0.05$). There were no significant differences between vegan diets for any of the calculated indices ($P>0.05$).

## Discussion and Conclusions

Differences in growth and feed utilization may be attributed to the nutritional composition of the plant-based diets, which, although rich in carbohydrates, were low in protein and lipids. Furthermore, despite the high feed intake observed in the fish fed vegan diets, the final protein intake was lower (0.046-0.060 g protein/day/fish) compared with the control diet (0.079 g protein/day/fish). An imbalance in the amino acid profile or any other nutritional deficiency may also have affected fish growth. On the other hand, selective feeding on certain ingredients in the vegan diets cannot be ruled out.

Protein and lipid levels were considerably lower in the vegan diets compared with the commercial diet, but not very different from those used by Luzzanna et al. (2005) and De Basis et al. (2012), where protein levels were similarto seed-based diets supplied in the present study. In their case, better growth results were obtained suggesting nutritional deficiencies in the vegan diets.

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**Table 1.** Macronutrient composition on a dry weight basis of the diets used.

| Feed | Control | Vegan | Vegan+Gi | Vegan+Ch | P |
|---|---|---|---|---|---|
| Moisture | 8.12±0.33a | 76.58±0.92b | 70.59±0.36c | 70.54±0.15c | (*) |
| Crude Protein | 52.59±0.25a | 20.14±0.57b | 21.44±1.21b | 18.42±0.43c | (*) |
| Crude Lipid | 15.89±0.13a | 1.28±0.19b | 7.52±1.14c | 5.37±1.62c | (*) |
| Ash | 8.76±0.02a | 1.23±0.17b | 1.95±0.17c | 1.97±0.19c | (*) |
| Total CH | 22.76±0.29a | 77.14±0.28b | 69.08±1.62c | 73.9±1.29d | (*) |
| Gross energy (KJ/100g) | 2239.34 | 1816.84 | 1952.32 | 1883.41 | |
| P/E (g/MJ) | 23.48 | 11.09 | 10.98 | 9.78 | |

*$P < 0.05$. Different letters indicate significant differences between diets.

**Table 2.** Growth and feed utilization indices for a commercial extruded diet and the three vegan diets tested in *Chelon auratus*.

| Indices | Control | Vegan | Vegan+Gi | Vegan+Ch | P |
|---|---|---|---|---|---|
| Wi (g) | 5.21±0.07a | 5.28±0.08a | 5.3±0.13a | 5.27±0.08a | (*) |
| Wf (g) | 8.71±1.01a | 5.85±0.18b | 6.07±0.39b | 5.95±0.12b | (*) |
| Wm (g) | 6.96±0.49a | 5.56±0.13b | 5.69±0.26b | 5.61±0.05b | (*) |
| WG (g) | 3.5±1.05a | 0.57±0.11b | 0.76±0.26b | 0.67±0.18b | (*) |
| AFR(g/d/fish) | 0.16±0.02a | 0.98±0.03b | 0.94±0.03b | 0.90±0.03b | (*) |
| SFR (%P/d) | 2.32±0.16a | 17.56±0.82b | 16.58±1.28b | 16.04±0.45b | (*) |
| AFRd(g/d/fish) | 0.15±0.02a | 0.23±0.01b | 0.28±0.01b | 0.26±0.01b | (*) |
| SFRd (%W/d) | 2.13±0.15a | 4.11±0.19b | 4.89±0.38b | 4.72±0.13b | (*) |
| AGR (g/d) | 0.09±0.03a | 0.01±0.00b | 0.02±0.01b | 0.02±0.01b | (*) |
| SGR (%P/d) | 1.27±0.30a | 0.25±0.04b | 0.33±0.10b | 0.3±0.08b | (*) |
| FE (%) | 53.30±8.43a | 1.46±0.32b | 2.04±0.74b | 1.87±0.49b | (*) |
| FEd (%) | 58.01±9.17a | 6.23±1.38b | 6.93±2.50b | 6.34±1.68b | (*) |
| FCR | 1.91±0.30a | 70.61±14.09b | 54.17±22.25b | 55.97±14.15b | (*) |
| FCRdCIs | 1.75±0.28a | 16.54±3.30b | 15.98±6.56b | 16.49±4.17b | (*) |

*$P < 0.05$. Different letters indicate significant differences between diets.

**Acknowledgements**

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# DAILY RHYTHMS OF BEHAVIOURAL RESPONSES TO ACUTE STRESS IN THE ROCKPOOL SHRIMP (*Palaemon elegans* RATHKE, 1837)

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## Introduction

During commercial shrimp farming, common practices often induce acute stress, triggering a range of behavioural and physiological responses. Decapod species respond to these stressors through changes at various biological levels, affecting their physiology, behaviour, and welfare in farming systems (Martins et al., 2012). Additionally, previous research has provided evidence of circadian variation in crustacean physiology(Louis & Emery, 2025). Palaemon elegans is an interesting experimental model in animal welfare research due to its wide distribution, commercial interest and ability to tolerate various environmental stressors. This crustacean species serves as a food source for other commercial species (Moronidae, Sparidae), is consumed directly, and consequently is being investigated as a new species in aquaculture. This study aims to characterize the daily rhythm in locomotor activity of the rockpool shrimp and investigate its behavioural stress responses, assessing the influence of the time of day. It provides a reference model for other commercially important shrimp species, facilitating the optimisation of management strategies in aquaculture and promoting sustainable practices in the shrimp industry.

## Methodology

A total of 32 shrimp with an average total length of 4.25 ± 0.14 cm were acclimated for 38 days and maintained in a 40 L aquarium under a 12:12 h light:dark (LD) cycle, fed once a day during the night. The aquarium was equipped with an infrared photocell to record the locomotor activity of the experimental subjects prior to the stress challenge and behavioural testing. After acclimation, 16 individuals were exposed to air for 60 seconds (stress group), either at ML (mid-light; n=8) or MD (mid-darkness; n=8). A bifactorial design was implemented with 4 subgroups: Control-ML, Control-MD, Stress-ML, and Stress-MD (n = 8 shrimp per subgroup). Following the stress challenge, two behavioural tests (open field and novel environment) were conducted. Control shrimp were also tested at the same time-points (ML and MD). Anxiety-related behaviours (i.e., immobilization, thigmotaxis, bottom-dwelling, erratic movement) and activity parameters (i.e., distance travelled) were evaluated using EthoVision XT® software. Locomotor activity recorded in the housing tank was analysed using the chronobiology software El Temps. For statistical analyses, when data met the assumptions of normality and homogeneity of variances, two-way ANOVA (ANOVA II $p < 0.05$) was used. When these assumptions were not met, the nonparametric Scheirer-Ray-Hare test was applied.

## Results

During acclimation, shrimp displayed a mostly nocturnal activity pattern, with 65% of their daily activity being recorded during the dark phase. In the open field test, two behavioural parameters showed significant differences between day and night. Thus, the thigmotaxis response was higher at MD (Fig. 1A; Scheirer-Ray-Hare, $p<0.04$), indicating that shrimp spent significantly more time at the periphery of the aquarium during the night. Similarly, angular velocity analysis revealed significantly more erratic behaviour at night (Fig. 1B; Scheirer-Ray-Hare, $p<0.001$). In the novel environment test, freezing behaviour was also significantly greater at night. In contrast, walking distance (Scheirer-Ray-Hare, $p<0.01$) and erratic movements (ANOVA II, $p<0.01$) were higher during the day.

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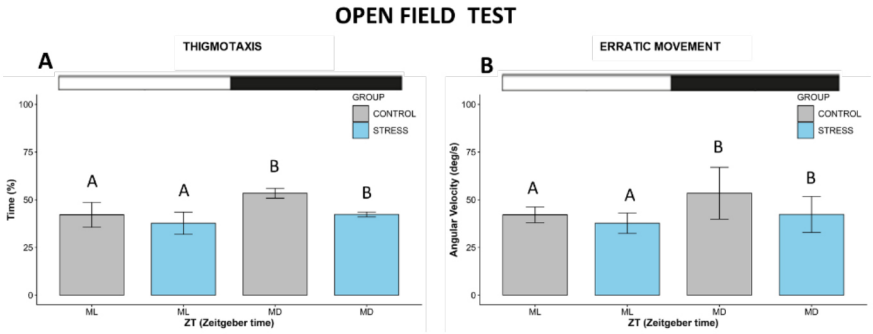


**Figure 1**. Thigmotaxis (A) and Erratic Movement behaviours during the Open Field Test at ML and MD in control (grey bars) and stressed (blue bars) shrimp. Data are presented as mean ± standard error, and superscript letters (A, B) indicate significant differences between time periods (ML and MD) ($p<0.05$).

## Discussion and conclusion

Our findings reveal that *Palaemon elegans* displays clear day/night differences in both locomotor activity and behavioural responses to acute stress. Shrimp were more active at night, and some anxiety-related behaviours such as thigmotaxis and freezing were more pronounced during the dark phase, suggesting higher stress sensitivity during their active period. However, in the novel environment paradigm, walking distance and erratic movements were higher following the stress challenge at ML, suggesting a shift in behavioural strategy depending on the time of day. While these results suggest a circadian modulation of stress-related behaviours, they remain preliminary and rather inconclusive due to the limited sample size and scope. Further research is needed to confirm these patterns across different environmental conditions and developmental stages. Even so, this study supports the potential of *P. elegans* as a chronobiological model and highlights the value of considering time-of-day effects in welfare assessments and aquaculture practices.

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# FROM BRAINWAVES TO WELFARE: ADAPTING EEG METHODOLOGIES TO ASSESS STUNNING EFFICACY IN SOLE SLAUGHTER

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## Introduction

Animal welfare represents a fundamental pillar in modern aquaculture, which aims to develop sustainable, ethical, and scientifically supported practices. In current farming practices, the slaughter phase is critical, as inadequate measures to minimise suffering during this process can compromise animal welfare. Current guidelines, therefore, underscore the need to use an appropriate stunning method that renders fish unconscious immediately prior to slaughter (OIE, 2019). In the case of sole, slaughter is typically carried out using traditional methods involving live chilling. However, compliance with welfare standards during this method remains uncertain. Consequently, sole farmers have expressed interest in identifying the most appropriate sole´s slaughter method from a welfare perspective. To determine a solid methodology, it is essential to conduct a rigorous evaluation of existing stunning methods. Traditional studies have evaluated stunning by assessing operational indicators based on clinical and behavioural responses (Morzel et al., 2003). However, these approaches have been demonstrated to be inconclusive as these indicators exhibit poor correlation with neurological activity (Bowman et al., 2019). Consequently, the recommended technique for assessing the efficacy of the stunning methods is electroencephalography, as it displays the effects of stunning at the brain level (OIE, 2019). This study aims to adapt existing electroencephalographic procedures to assess the effectiveness of stunning in sole.

## Materials and methods

The present study used commercial-sized sole (*Solea senegalensis*), weighing between 500 to 800 g, to develop a methodological protocol for electroencephalogram (EEG) recording. An anatomical comparison with turbot was performed using a computed tomography (CT) scan to identify the position of the electrodes and adapt the EEG methodology developed for turbot (Ramirez-Rodriguez et al, 2024). To record the EEG signal, each fish was anaesthetised with MS-222 (80 mg/L) to facilitate the surgical placement of stainless-steel electrodes. After anaesthetising the fish, baseline EEG activity and visual evoked responses (VERs) were recorded using a strobe light stimulus. Spectral EEG parameters, including total power (Ptot), alpha-beta power (Pαβ), median frequency (F50), and spectral edge frequency (SEF), were obtained by power spectrum analysis using Spike2 software. VERs were analysed by averaging light-induced responses over one-second windows.

## Results

Our study adapted the EEG protocol from turbot to record brain activity in the sole. The use of an anatomical comparison via a CT scan facilitated the identification of analogous skull regions to those identified by our group for EEG recording in turbot. This allowed us to apply previously developed methodologies with only minor anatomical adjustments. Spectral analysis of the EEG baseline signal demonstrated the feasibility of analyzing brain activity. The spectral parameters were sensitive to alterations in brain activity associated with stunning procedures. Furthermore, this study demonstrated the feasibility of detecting VERs in the sole using strobe light stimulation, thereby enabling the evaluation of its visual sensory perception capabilities.

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## Discussion

Our study represents a significant advance in developing tools to support more conclusive decision-making when assessing the effectiveness of existing stunning methods for slaughtering sole. This study demonstrated the technical feasibility of adapting the EEG recording protocol originally developed for turbot to sole. This adaptation enabled the successful acquisition and analysis of brain activity in sole, thereby expanding the applicability of neurophysiological tools within the field of fish welfare research. Anatomical comparison via CT scan is crucial for identifying homologous cranial regions and adapting electrode placement, thereby facilitating the accurate and consistent acquisition of EEG signals. The ability to record a reliable EEG signal facilitates the estimation of classical spectral parameters, including total power (Ptot), alpha-beta power (Pαβ), median frequency (F50), and spectral edge frequency (SEF) parameters (Tonner & Bein, 2006). This methodology demonstrates its efficacy in monitoring brain activity in the sole, thereby substantiating its potential to provide objective indicators of sentience. Notably, the present study confirmed the feasibility of detecting VERs in sole. The ability to identify the presence of VER strengthens the robustness of the EEG recording methodology for use in assessments of stunning efficiency. This is because it can provide an indicator that has been described as reliable for this type of evaluation in other fish species (Hjelmstedt et al., 2024). In summary, our findings demonstrate that the adapted EEG methodology provides direct insight into brain activity and enables objective indicators of sensitivity in the sole to be identified. This methodological advancement provides the basis for a decision-making support tool designed to promote animal welfare during the industrial sole slaughter. Implementing EEG-based assessments offers a scientifically robust approach for evaluating the effectiveness of stunning methods. This supports the development of more ethical, evidence-based protocols in modern aquaculture.

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# ONTOGENESIS OF THE RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINE AND ITS STEM CELL NICHE FROM LARVAL TO JUVENILE

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**Introduction**

The transition from endogenous to exogenous feeding characterizes early developmental stages of farmed fish and it is often associated with high mortality rates, resulting in economic loss and reduced animal welfare[1,2]. The gut and its stem cell niche, as major actors in nutrient absorption and drivers of epithelial development respectively, play a key role in this delicate event[3]. Therefore, expanding the current understanding of intestinal ontogenesis could improve farming efficiency. In this study, we investigated the intestinal morphological and cellular changes across three early developmental stages of rainbow trout (*Oncorhynchus mykiss*) - larval, parr, and juvenile – particularly focusing on the cellular populations of the intestinal stem cell niche.

**Materials and methods**

Whole larvae (20 dph) and intestinal samples from parr (50 dph) and juvenile (12 months) rainbow trout were collected and processed for histological purposes. Briefly, we used: (i) hematoxylin and eosin to assess the general tissue morphology; (ii) Masson's trichome to highlight the compactum layer of the *submucosa*; (iii) Alcian blue (pH 2,5) and Periodic Acid Schiff, used in combination, to detect mucins-secreting cells. Proliferating intestinal cells were identified through immunofluorescence of proliferating cell nuclear antigen (PCNA); in situ-hybridization was used to detect the expression of *sox9* and *pdgfrα* as specific markers of intestinal stem cells and telocytes - a stromal cell population regulating stem cell differentiation [4,5]according to the frequency and distribution of specific cell types, the rainbow trout (RT.

The transition to the parr stage was marked by a more structured morphology with intestinal folds acquiring a finger-like phenotype and an increased complexity of the *submucosa*. At this stage, a fully developed epithelial stem cells niche restricted to the fold's base became evident (Fig 2). Moreover, at this stage, enteroendocrine cells appeared scattered along the epithelium.

At the juvenile stage, the *mucosa* acquired its definitive configuration with long finger-like folds and a fully developed *submucosa* consisting of the three distinctive layers: the two granulosum and the thick collagen compactum layer located in between.

**Conclusion**

The timing of these events suggests that telocytes play a key role in triggering and guiding the formation of a well-defined stem cell niche and that its differentiation is largely completed by the parr stage.

These results expand and integrate the current understanding of the ontogeny of the rainbow trout intestine, providing novel insights into this delicate phase. The definition of the accurate timing of the detailed intestine development may help in optimizing feeding strategies and improving animal welfare, limiting possible economic losses.

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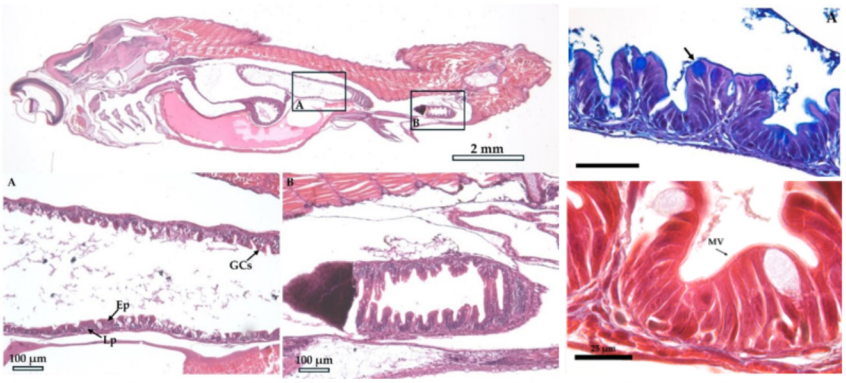


Figure 1: representative images of HE, PAS – AB staining and Masson's thricrome showing the general morphology of rainbow trout larvae (Ep: epithelium, Lp: lamina propria, GCs: goblet cells), the presence of physiological mucus protection (arrows), and microvilli (MV).

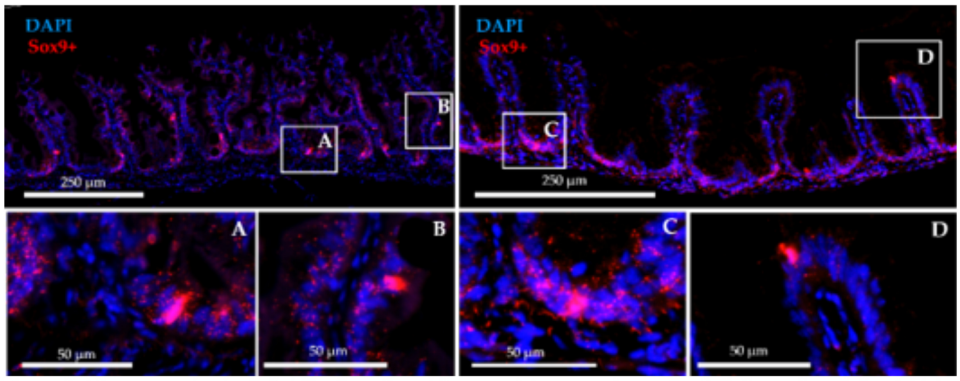


Figure 2: Fluorescent in situ hybridization of *sox9* in par rainbow trout's proximal (left panel) and distal (right panel) intestine. Slender *sox9*$^{+}$ cells were detected at the folds base (A-D) and at the folds apex (B-D) in both intestinal regions.

**Acknowledgements:**
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# COMPARATIVE ANALYSIS OF THE CREATINE TRANSPORTER SLC6A8 IN RAINBOW TROUT (*Oncorhynchus mykiss*): INSIGHTS INTO VARIANT-SPECIFIC EXPRESSION AND FUNCTION IN TELEOST FISH

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**Introduction**

The creatine system in vertebrates is an important cellular energy buffer, maintaining high-energy phosphate bonds that quickly replenish adenosine triphosphate (ATP) when needed. This is especially important in tissues with a high energy demand like the brain, heart or muscle. Creatine can be gained via nutrition or it can be synthesized intrinsically through a two-step mechanism catalyzed by the enzymes glycine amidinotransferase (GATM), guanidinoacetate N-methyltransferase (GAMT) and creatine kinases (CKs). The uptake of creatine from the bloodstream into cells is mediated by the sodium- and chloride dependent creatine transporter 1 (CT1), which belongs to the solute carrier 6 (SLC6) transport family of secondary active $Na^+/Cl^-$ co-transporter (Kristensen et al., 2011).

Due to their different habitats, fishes have developed different metabolic strategies compared to other vertebrates. We have recently shown that teleost fish and mammals differ in regard to their creatine systems. The findings indicate the presence of a muscle-specific creatine synthesis in fish (Borchel et al., 2019). Based on these differences, the present study was aimed to further enhance the knowledge on the transportation of creatine in a teleost model species. Hence, we characterized the *SLC6A8* genes encoding the creatine transporter CT1 in rainbow trout *Oncorhynchus mykiss*. Structural and functional properties in terms of evolutionary conservation, as well as their role as creatine transporter in a salmonid cell model were investigated by complementary molecular techniques.

**Material & Methods**

Database searches identified two entries in the NCBI (National Center for Biotechnology Information; http://blast.ncbi.nlm.nih.gov/) database: rainbow trout *SLC6A8* gene variants *SLC6A8a* (XM_021566996) and *SLC6A8b* (XM_036947549). Structural analysis of functional domains and the prediction of the transmembrane topology of the encoded protein sequences were conducted using the InterProScan (https://www.ebi.ac.uk/interpro/search/sequence/) and the Protter (https://wlab.ethz.ch/protter/start/) tools. 3D-modelling and visualization was performed by SWISS-MODEL (https://swissmodel.expasy.org/) and UCSF Chimera (http://www.rbvi.ucsf.edu/chimera). The evolutionary relationship of the gene variants and the corresponding orthologous genes from other teleosts and higher vertebrates was investigated by synteny analysis using the Genomicus database (v98.01 http://genomicus.biologie.ens.fr/genomicus) and the construction of a phylogenetic tree for the SLC6 family in MEGA 11 software by using the Neighbour-Joining method including 1000 bootstrap replicates and the Poisson correction method.
Expression of creatin system genes *SLC6A8a*, *-b*, *GATM*, *GAMT*, and *CKM* in rainbow trout was quantified in different tissues and the salmonid cell line CHSE-14 (Sigma-Aldrich) by quantitative PCR on the LightCycler96 system (Roche Diagnostics) and in publicly available RNA-Seq data from BioProject PRJNA638521.

Live-cell imaging was performed using the confocal microscopy LSM 780 (Carl Zeiss Microscopy) to determine the subcellular localisation of constructed GFP-SLC6A8a - and Plum-SLC6A8b or GFP-SLC6A8a and Plum-SLC6A6 fusion genes. Creatine uptake in pre-incubated CHSE-214 cells was determination in extracts of white-muscle tissue.

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**Results and Discussion**

The *SLC6A8a* and *SLC6A8b* gene variants show variant-specific expression patterns (Figure a). Both exhibit the highest transcript level in the heart. Despite this similarity, *SLC6A8a* significantly exceeds the expression of *SLC6A8b* in the heart, brain, spleen, head kidney and gill tissue, while *SLC6A8b* is predominantly expressed in the muscle and liver. Moreover, we detected clear differences in the expression of the central creatine system genes *GATM*, *GAMT* and *CKM* in the heart and skeletal muscle (Figure b) suggesting an overall different creatine metabolism between both tissues in rainbow trout.

The pre-incubation of the CHSE cells with creatine resulted in its significant increase in concentration in the cells from 0.52 mM to 0.83 mM (Figure c), indicating its carrier-dependent transport of creatine across the cell membrane. In line with this, we verified the cellular localisation of rainbow trout proteins SLC6A8a and SLC6A8b in the cell membrane (Figure d-f).

The present study extends the knowledge of the creatine transporter SLC6A8 in rainbow trout and supports the previously reported differences in the creatine system of mammals and teleost fishes. However, further functional analyses are required to deepen the knowledge on the variant-specific membrane transport of creatine by SLC6A8a and SLC6A8b in fish.

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# IMPROVING STRESS RECOVERY IN ATLANTIC SALMON (*Salmo salar*) WITH FUNCTIONAL FEED

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## Introduction

In intensive production of Atlantic salmon, the fish are subjected to various handling procedures such as transport, vaccination, and mechanical treatments for sea lice. These events induce acute stress in the fish. Additional stressors include sudden changes in water quality or environmental changes during the transition from freshwater to seawater. Stressed fish exhibit reduced disease resistance and diminished appetite [1, 2], leading to decreased growth and economic losses for fish farmers. A recent study estimated that a single mechanical treatment against sea lice could result in a 200-gram growth loss per fish [2]. Feed that enhances appetite following a stressful treatment can improve growth and robustness, ultimately reducing the time required to reach the desired market weight. To accurately access feed efficacy, it is essential to move beyond controlled lab-scale trials and consider real-world challenging environments.

## Materials and methods

A challenge model was developed to emulate the handling of Atlantic salmon prior to mechanical delousing in well boats. The handling procedure consisted of mild crowding for 1.5 hours, followed by netting, sedation, individual weighing, and manual delousing. This model was validated through a nine-month feeding trial conducted at LetSea (Dønna, Norway), which compared stressed and non-stressed treatments among the salmon. Throughout the trial, the handling procedure was performed four times. This challenge model has been used in assessing functional feeds at LetSea. The test feed in this trial contains additional magnesium and specific plant raw materials, which are hypothesized to enhance appetite. The primary objective was to mitigate stress-related growth loss in Atlantic salmon post-handling. The experimental feed was administered two weeks preceding, and during the week of handling.

## Results

The challenge model was verified by a significant stress response shown as increased levels of cortisol in fish scales, serum biomarkers such as the secondary stress response parameters lactate, glucose, magnesium, calcium and potassium, and altered expression of stress-related genes in the liver of stressed fish compared to the non-stressed control treatment. In addition, the cardio somatic index (CSI) and heart length and roundness were significantly affected by the stress treatment, with unstressed fish having lower CSI and wider and rounder hearts, and stressed fish had increased skin hemorrhages and snout wounds.

During the feeding trial the fish grew from approximately 1.5 kg to 4.3 kg. Comparing stressed control treatments with stressed functional feed treatments, we have seen no significant differences in cortisol levels in scales or stress response serum markers. This indicates that the fish fed the functional feed had a normal adaptive stress response. Additionally, we have seen an increase in appetite (increased feed intake) and growth after the handling events for fish fed the functional feed, as evidenced by higher TGC (thermal growth coefficient). Despite the stress induced by the challenge model, the fillet color (pigmentation) was improved reflecting the increased feed intake.

## Conclusion

The findings of the current study underline the importance of addressing stress and growth in fish through scientific interventions. The challenge model tested effectively simulates the handling of Atlantic salmon prior to mechanical delousing operations and has been verified through extensive feeding trials. The new functional feed significantly improves appetite and growth in salon following stress-induced handling procedures.

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# OMEGA-3 FATTY ACID LIPOGENIC FLUX IN MICROALGAE – USING DEUTERATED WATER TO GROW *Munda aquilonaris*

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The consumption of essential fatty acids, namely omega-3, has been increasingly linked to major health benefits. Currently the main dietary source of these highly valuable fatty acids are small oily fish and their derivatives. Their metabolic capabilities to synthesize these compounds is limited, as is in most vertebrates, and their rich composition derives from biomagnification processes along the marine trophic chains. These originate in microalgae, the primary producers of such high-value polyunsaturated fatty acids (PUFA). As such, understanding the metabolic pathways and lipogenic fluxes of these organisms is extremely relevant particularly if the goal is to optimize and maximize their yields.

Deuterated water ($^2H_2O$) has systematically provided reliable lipid biosynthetic flux estimates, validated in several different animal models. The incorporation of deuterium ($^2H$) into cells follows well-defined metabolic steps and positional $^2H$-enrichments can be traced by deuterium Nuclear Magnetic Resonance ($^2H$-NMR). In order to validate its utilization, this study aimed to determine if $^2H$-enrichment of the medium interferes with microalgae growth, while assessing the effectiveness of fatty acid $^2H$-labelling in microalgae. To achieve this, *Munda aquilonaris* ACOI 2424 was cultivated in three distinct $^2H$ concentrations, while growth was monitored through cell counting. Lipids extracted from the resulting biomass were analyzed using NMR, acquiring both $^1H$ and $^2H$ spectra. The results showed that $^2H$ was successfully incorporated into the metabolic pathways enabling the distinction of the *de novo* omega-3 fatty acid synthesis.

These findings pave the way for innovative metabolic studies using this stable isotope to trace omega-3 lipogenic fluxes in microalgae. This could enhance the production of essential fatty acids and uncover new possibilities for applications in human and animal nutrition.

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# PEANUT PEEL AS A SUSTAINABLE ADDITIVE AND INGREDIENT FOR GILHEAD SEABREAM JUVENILES (*Sparus aurata*)

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## Introduction

The increasing global population poses a major challenge to the sustainable production of seafood. Although plant-based protein sources are commonly used in aquafeeds, their sustainability is limited by competition with the human food supply and the high carbon footprint associated with their production and transport. In this context, the circular economy concept, particularly emphasized by the European Union's Farm to Fork strategy, promotes the valorization of agro-industrial by-products. These by-products are generated in large quantities and are often discarded, thereby contributing to environmental impact. Europe imports around 800.000 tons yearly, which corresponds to 32.000 tons of peanut peel generated by-products (FAOSTAT, 2022; Manrich et al., 2022). Due to their composition, these by-products may be used as feed additives or alternative ingredients for aquafeeds. Among these, peanut peel as a major by-product of the peanut butter industry, typically discarded, representing an additional cost to the industry. Despite being undervalued as a feed ingredient, peanut peel contains moderate protein and lipid levels and a high concentration of bioactive antioxidant compounds that may promote fish wellbeing. To the best of our knowledge, this is the first study to evaluate the use of peanut peel in aquaculture. Therefore, this study aims to evaluate the apparent digestibility coefficients (ADCs) of the peanut peel by-products and to assess the effects of its dietary inclusion at increasing levels on the zootechnical performance of juvenile gilthead seabream (Sparus aurata).

## Materials and methods

The apparent digestibility of peanut peel by-products (PP) ingredient was evaluated. Two diets were formulated to determine ADCs; a control diet similar to a current commercial diet, and a test diet, containing 30% PP and 70% of the control diet. Apparent digestibility coefficients (ADCs) of dry matter, protein, lipid, and energy for both diets and the PP ingredient were determined according to Bureau et al. (1999). The digestibility trial was performed in a RAS system with 6 fiberglass tanks (60 L) equipped with fecal sedimentation columns. Triplicate groups of 15 gilthead seabream juveniles (IBW: 50g) were fed twice a day, and feces were collected daily, before the morning meal, for 30 days.

A growth trial was also conducted to evaluate the zootechnical effects of dietary inclusion of PP. Five isoproteic (44%) and isolipidic (18%) diets: a control diet similar to a current commercial diet, and 4 diet including an increased level of PP. Two diets included 0.5% and 1% (0.5PP and 1PP), to evaluate PP as functional ingredients, and 2 other diets including 5% and 10% peanut peel (5PP and 10PP), replacing up to 56% of soybean meal to evaluate PP's potential as an alternative ingredient. The growth trial was conducted for 12 weeks in a RAS system maintained at 24 ºC. Triplicate groups of 15 gilthead seabream juveniles (initial body weight: 36g) were fed until apparent satiation, six times per week. At the end of the trial, fish's growth performance, feed intake, feed efficiency, protein efficiency ratio, nitrogen, and lipid retention were evaluated. At the end of the trial, 3 fish per tank were sampled, and liver and intestine were collected for oxidative stress analysis and lipid peroxidation, and muscle for lipid peroxidation analysis.

Digestibility data were analyzed using one-way ANOVA. Growth performance data were evaluated by polynomial contrast analysis and one-way ANOVA, followed by Tukey's post hoc test. A significance level of $p < 0.05$ was used for all statistical analyses.

## Results

Apparent digestibility coefficients (ADCs) for dry matter, protein, lipid, and energy were statistically similar between the control and peanut peel diets, indicating that the inclusion of up to 30% peanut peel in aquafeeds does not compromise overall nutrient digestibility. The apparent digestibility coefficient for protein in peanut peel was approximately 38.5% ± 20.1, whereas lipid digestibility reached 73.5% ± 6.8.

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During the growth trial, mortality was low and not affected by the dietary inclusion of PP. Weight gain, daily growth index, feed intake, feed efficiency, protein efficiency ratio, and lipid retention were not influenced by increasing levels of PP. Nitrogen retention was also unaffected by PP inclusion; however, a quadratic tendency was observed, with increased retention in diets containing 0.5%, 1%, and 5% PP compared to the control diet, and a decrease at the 10% inclusion level. Whole body dry matter, lipid, and ash contents were not influenced by PP inclusion. However, whole-body protein content showed a quadratic trend to increase in the PP included diets compared to the control. Visceral and hepatosomatic indexes were not affected by the inclusion of PP.

**Conclusion**

Peanut peel showed moderate digestibility as an ingredient, but when included at 30% in the diet, its digestibility was comparable to that of the control diet. The dietary replacement of soybean meal by PP up to 10% (representing a replacement level of 56%) did not affect growth performance, feed utilization or whole-body composition. Oxidative stress and lipid peroxidation results are being analysed and will be presented.

These findings highlight the potential of peanut peel as a sustainable alternative ingredient in aquafeeds and open new avenues for future research on its functional and nutritional applications in aquaculture.

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# ARCHIMEDES SCREW HELPS OPTIMIZE FISH CATCHING AND WELFARE

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## Introduction

Fish used in aquaculture often need to be moved between tanks or sites, especially during the pre-slaughter period. However, the effects of screw pumps *vs.* suction pumps on welfare and flesh quality are not well understood. Research has indicated that vacuum pumps can cause damage to pelvic fins and increase stress responses in salmonids, potentially leading to higher mortality rates (Espmark *et al*., 2016). In contrast, the Archimedes screw is considered "fish-friendly" due to the absence of sudden pressure changes and hydraulic shear forces (Þórðarson *et al.*, 2022). An additional factor that has yet to be explored is the interaction between water temperature and the fish's response to catching methods. This study compared the impacts of two mechanized methods on stress indicators and quality parameters in rainbow trout (*Oncorhynchus mykiss*) in winter (WI) and summer (SU).

## Materials and methods

A total of 240 trout with an average weight of 248.9 ± 46.2 g were captured using nets (N), a screw (A), or a vacuum pump (V), in WI (10°C) and SU (17ºC), ensuring the quickest yet gentlest movements. Less than a minute later, they were stunned using a dry stunning method. In order to assess animal welfare and flesh quality, several plasma parameters were measured (cortisol, lactate, non-esterified fatty acids, and creatine phosphokinase), as well as skin colour, and muscle pH.

## Results & Discussion

At 0 hours *post-mortem*, pH values for A-WI were higher than in all other cases, with values for N and V in WI also higher than those observed in SU, where the metabolic effort appeared to be lower. Increased effort can lead to increased lactate levels and subsequent pH drops (Milligan, 1996). At 24 hours post-mortem, pH levels showed treatment interaction, which was highest for N-SU, while N-WI had the lowest pH. Additionally, A-WI had lower pH than V. This could be due to the higher energy demand of method V, which may result in greater stress. Cortisol levels varied by catching method, with the highest levels observed in V, suggesting that the suction pump may impose higher stress (Þórðarson *et al*., 2022), followed by A and N. Lactate was influenced by the interaction of catching method and seasonality, with N in WI showing lower levels compared to other groups, reflecting reduced movement due to colder temperatures; while A-SU exhibited higher levels than N and V in SU, suggesting acute effort in A and the hypothesis that method V may impose greater stress, as indicated by its lower lactate levels. NEFA was higher in fish caught with V-SU relative to A and V in WI, however, its levels were lowest for V-WI compared to A-SU and N at both seasons. The consistency in NEFA levels across seasons for N and A suggests a reduced stress response compared to V. The CPK-WI levels were consistently higher than SU across all catching methods due to the slower metabolism, with A-WI showing significantly elevated levels compared to the other cases, highlighting the increased energy demand associated with physical exertion (Liquin *et al*., 2016) as in lactate. Skin lightness (L*) was higher for N-WI compared to the other cases, and with lower values observed for V-SU than in WI, which could be attributed to higher temperatures during SU or the physical effort required by the catching method during WI (Iger *et al*., 2001). Higher yellowness (b*) and chroma (C*) values were observed in N-SU and in V-WI, while lower values were found in A in WI and SU and in V-SU. N-WI also had lower b* and C* values compared to SU. These findings suggest that method V may be more stressful than method A, similarly to Erikson & Misimi (2008). Hue (h*) was affected by the interaction, with A in WI showing lower values than N-SU and V-WI, possibly linked to the higher activity associated with this method, suggesting h* as an indicator of trout exertion.

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## Conclusions

Based on our trial, the Archimedes screw appeared to be more product and welfare friendly than the suction pump for trout harvesting, despite being more demanding a higher metabolic effort. However, it is essential to ensure that water temperatures remain within the optimal range for trout during pumping/movement.

## Acknowledgement

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# FROM ISOLATION TO APPLICATION: THE ROLE OF *Cetobacterium* sp. C33 IN NILE TILAPIA

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Intestinal microbiota plays a crucial role in fish health, with anaerobic bacteria such as *Cetobacterium,* emerging as key contributors to gut homeostasis. In this study, we report the isolation, genomic characterization, and probiotic potential of *Cetobacterium* sp. C33, a strain obtained from the intestinal tract of Nile tilapia (Oreochromis niloticus) under anaerobic conditions.

In vitro analyses demonstrated C33's adaptability to gastrointestinal conditions, including survival in bile salts (0.3%) and low pH. Whole-genome sequencing and annotation revealed a unique genetic profile distinct from closely related species, such as *Cetobacterium somerae* and *Cetobacterium ceti*. The genome harbors genes linked to antibacterial activity, intestinal adhesion, and immune modulation, reinforcing its potential as a probiotic. Further comparative genomics confirmed its phylogenetic divergence from previously characterized *Cetobacterium* species. Also, the absence of toxin-related genes and antibiotic resistance further supports its safety as a probiotic candidate.

To validate its functional application, an in vivo trial was conducted by feed administration of *Cetobacterium* sp. C33 at 1 × 10□ CFU/g to tilapia fingerlings for five days. Results revealed 100% survival, successful intestinal colonization, modulation of the gut microbiota, and immune response regulation, as evidenced by transcriptomic analysis of the head kidney that revealed upregulation of immune genes such as claudin-8-like Perforin 1, E3 ubiquitin among others.

# PRODUCTION OF ALTERNATIVE PROTEINS FROM AGRI-FOOD BY-PRODUCTS THROUGH MICROALGAE CULTIVATION (PROALGAE)

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**Introduction**

The PROALGAE project will explore the feasibility of producing alternative protein through microalgal biomass grown on agri-food industry byproducts (i.e. process effluents), within a circular economy context. Thus, it is built on the following hypotheses: i) specific agri-food by-products can be valorized by recovering their nutrients through the growth of a high-value microalgal biomass, and ii) microalgal biomass production using agri-food industry by-products is a sustainable and climate change resilient alternative for protein production that can be used for food and feed.

**Materials and Methods**

The research will focus on specific agri-food by-products, i.e. cheese whey, aquaculture, fish canning, beverage production and hydroponic culture effluents. Firstly, the feasibility of growing target microalgae species in those effluents will be tested, aimed at obtaining the maximum productivity of specific species feasible to be used as feed or food, as well as achieving the desired treated effluent quality. Then, the microalgal biomass will be characterized, for monitoring the production of compounds of nutritional interest and food-and-feed safety issues. Finally, the microalgal biomass will be used for the production of food and feed products. The characteristics of the food products will be analysed in order to assess the acceptability of these bioproducts as alternative protein source, while the feed products will be tested as alternative feed ingredients. In addition, the economic, environmental and social sustainability of the proteins production from microalgae grown in agri-food by-products will be assessed, and the potential production will be evaluated through model simulations and market strategy surveys, with the objective of assessing their potential industrial application prior to the industrial scale production.

**Current Status**

Currently different microalgae strains have been grown in different agri-food effluents. These screenings showed potential of growing *Nannochloropsis gaditana* in dairy by-products in particular in diluted whey and digestated whey. The optimal combination have been scalated to 50 L to prove the continous production of microalgae biomass while achieving wastewater treatment of the effluent. On the other side, *Scenedesmus* sp. cultivated in brewery effluents has shown to outperfom *Chlorella* sp. and *Spirulina* sp. The strain has been scalated to 4.5 L photobioreacors and grown in semicontinous operation during six weeks.

**Future directions**

The microalgae biomass will be characterized for protein and pigments content, nutritional properties, biomass composition and physical characteristics. Once characterized it will be used as ingredients in aquafeed for turbot (*Scophthalmus maximus*) culture: a control diet (CT) without microalgae, and three diets (M5, M10 and M20) with 5%, 10% and 20% microalgae, respectively. Samples of size and weight of all the fish will be carried out monthly after fasting 24 hours, to obtain the growth data. Fish growth performance will be analysed in terms of survival (S, %), weight gain (WG, g), percentage weight gain (WG, %), specific growth rate (SGR, % day -1), Fulton's condition factor (k) and hepatosomatic index (HI). After culture, the samples of the intestine for microbiota analysis will be collected, namely muscle and liver for the study of metabolites, and carcasses for proximal composition analysis. For the intestinal microbiome study16S rRNA sequencing analysis of bacterial DNA will be used.

# LC-PUFA BIOSYNTHETIC CAPACITY OF THE POLYCHAETE *Heduste diversicolor:* CULTIVATION ON SIDE STREAMS FOR CIRCULAR AQUEFEED APPLICATION

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## Introduction

The rapid growth of aquaculture has prompted the exploration of sustainably sourced ingredients that, like traditional marine ingredients as fishmeal and fish oil, are rich in the essential long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFAs) eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3). Biomasses derived from aquatic invertebrates, particularly polychaetes, have emerged as promising LC-PUFA-rich ingredients for aquafeeds [1]. Many aquatic invertebrates have the capacity for endogenous production (biosynthesis) of LC-PUFAs, with complete sets of key enzymes termed methyl-end (or "omega") desaturases (ωx), front-end desaturases (Fed), and elongases (Elovl) [2]. A unique trait of the LC-PUFA biosynthetic pathways from invertebrates including polychaetes, is the occurrence of ωx, enzymes that enable the *de novo* biosynthesis of PUFAs and, from them, n-3 LC-PUFAs [3]. This metabolic capacity, combined with their detritivorous feeding habits, makes polychaetes ideal candidates for Circular Economy approaches, where low-value, n-3 LC-PUFA-poor side streams from bioindustries can be recycled into high-value, n-3 LC-PUFA-rich biomass. The aim of the present study was to report on results collected from the molecular and functional characterisation of the LC-PUFA biosynthesising desaturases and elongases from the nereid polychaete *Hediste diversicolor*. Furthermore, due to the capacity of polychaetes to modulate the LC-PUFA biosynthesis in response to environmental factors, we carried out an *in vivo* feeding trial aiming to assess the effects of temperature and salinity on LC-PUFA biosynthesis in *H. diversicolor* juveniles fed on diets composed of side streams from the aquaculture and biogas industries.

## Materials and Methods

The molecular characterisation of desaturases and elongases involved in the *H. diversicolor* LC-PUFA was conducted by determining the phylogeny of the encoded enzymes. Searches for relevant sequences were performed in both public and non-public genomic/transcriptomic databases. Functional characterisation of the *H. diversicolor* desaturases and elongases was done by heterologous expression of their coding regions in brewer's yeast (*Saccharomyces cerevisiae*). Conversion of the enzymes was determined by analysing the proportion of the fatty acid substrate converted to the corresponding product(s). To evaluate the influence of temperature and salinity on LC-PUFA biosynthesis, an *in vivo* feeding trial was conducted using *H. diversicolor* reared on side stream-based diets. Polychaetes were cultured for 28 days along a 5-step temperature and salinity gradient ranging from 7.7 to 17.9 °C and 5 to 40 psu, respectively. Growth, survival and fatty acid (FA) profiles of the worms were analysed.

## Results and Discussion

Molecular and functional analyses revealed that *H. diversicolor* possess three elongases (Elovl2/5, Elovl4 and Elovl1/7) capable of elongating $C_{18}$ $C_{20}$ and $C_{22}$ PUFA substrates, and two front-end desaturases (Fed1 and Fed2), with Δ5 and Δ6/Δ8 desaturase activity, respectively. This enzymatic capacity is shared with other invertebrates such as molluscs, echinoderms and annelids [2]. Collectively, these enzymes, along with the two methyl-end desaturases reported by [4], confirm that *H. diversicolor* has complete pathways to biosynthesise LC-PUFAs, including ARA and EPA (Fig. 1). However, the data collected suggest that *H. diversicolor* is unable to biosynthesise DHA. Regarding the regulation of LC-PUFA biosynthesis by temperature and salinity, our findings showed that the FA composition of *H. diversicolor* remains relatively stable, regardless of the temperature conditions. Nevertheless, low salinity (5 and 15 psu) was associated with increased levels of n-3 LC-PUFAs primarily driven by EPA, in the lipids of the polychaetes. These results support that the activities of the LC-PUFA biosynthetic enzymes (desaturases and elongases) characterised in yeast, appear to be operative *in vivo* according to the high levels of LC-PUFAs found in the lipids of *H. diversicolor* fed on diets with very limited LC-PUFAs. Overall, our findings underscore the biotechnological potential of *H. diversicolor* for generating high nutritional value biomass for aquaculture, following Circular Economy principles.

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# REPLACEMENT OF DIETARY FISHMEAL ON THE GROWTH OF JUVENILE YELLOWTAIL, *Seriola lalandi* IN A RECIRCULATING AQUACULTURE SYSTEM

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Fishmeal is typically the main protein ingredient when formulating a diet for carnivorous fish due to its high protein content, good digestibility and balanced essential amino acid profile. Issues around the sustainability and high cost of fishmeal have driven research to reduce its inclusion and replacement using alternative animal protein sources. Two feeding trials were conducted to determine the effect of dietary protein and fishmeal replacement on survival, growth and feed conversion ratio (FCR) of juvenile yellowtail, *Seriola lalandi* in a RAS. The first experiment investigated four formulated iso-calorific diets containing different dietary protein levels (38%, 44%, 50% and 56%) for 49 days. Dietary protein level did not have an effect on survival of juvenile yellowtail. In terms of growth performance, specific growth rate (SGR) (ANOVA: $F(3,8) = 10$, $p = 0.003$) and weight gain ($F(3,8) = 10$, $p = 0.003$) was greater in fish fed 56% protein compared to fish fed 38% protein but there were no differences between the 56%, 50% and 44% protein diets. There were no differences in protein efficiency ratio (PER) (1.28±0.32- 1.6±0.5) between the four diets. FCR decreased with increasing protein level with fish being fed the 56% protein yielding the lowest FCR (1.26±0.09) compared to fish fed 38% protein (1.76±0.2) ($F(3,6) = 13$, $p = 0.003$) however there was no difference between the 44%, 50% and 56% protein levels.

A second experiment investigated alternative animal protein sources using the 44% protein diet from Experiment 1. Four experimental diets were formulated to be iso-nitrogenous (44% protein) and iso-calorific (19.50 Mg/Kg). Diets included (i) a control diet with 100% fishmeal as the only protein source (the same formulation as used in Experiment 1), (ii) 67% fishmeal, (iii) 33% fishmeal and (iv) 0% fishmeal with the balance of protein being made up in diets 2-4 of chicken meal, blood meal and soyabean meal in similar proportions. Different fishmeal levels in the diets did not have an effect on the survival on juvenile yellowtail. Specific growth rate was however greater (0.50 ±0.05-0.54±0.10) for fish fed the 67% and 100% fishmeal diets (One-Way ANOVA, $F = 6.27$ $df=3$, $p = 0.017$) compared to fish fed 0% fishmeal (0.16±0.16). There were however no differences in SGR between fish fed 33%, 67% and 100% fishmeal (0.46±0.17-0.54 ±0.10). Diets containing fishmeal had FCR's which were significantly lower than the diet with no fishmeal at all ($p = 0.001$). Fish fed diets having 67% and 100% fishmeal had low PER (0.44±0.11-0.45±0.11) ($p = 0.026$) compared to fish fed 0% fishmeal (0.11±0.10). The results suggest that a 44% protein diet is optimal and that full replacement of fishmeal (0% fishmeal diet) is not recommended as it produced the worst SGR, FCR and PER. The remaining diets were statistically similar suggesting that 33% fishmeal diet produces equivalent growth, FCR and PER as the 67% and 100% fishmeal diets. However, as there were similarities in SGR and PER for 0% and 33% fishmeal diets it is recommended that future experiments possibly focus on the 10% - 50% fishmeal component to find the optimal breakpoint for the various growth and feeding indicators while reducing diet cost and the reliance on fishmeal.

# A NOVEL EX VIVO MODEL TO STUDY WOUND HEALING IN FISH SKIN

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## Introduction

Skin wounds are a leading welfare concern and driver of mortality in salmonid aquaculture. However, the earliest stages of repair are difficult to track *in vivo*. Most existing wound-healing studies in fish rely on *in vivo* sampling at discrete time points, which limits resolution of the earliest repair processes. Our approach addresses this gap by providing a continuous, real-time measure of epithelial barrier restoration under controlled *ex vivo* conditions, using the Ussing chamber method.

Ussing chamber technique help to measure the electrophysiological properties from an epithelium *ex vivo*. The epithelium is placed between two chamber halves and covers a small aperture, through which solute and water transport and diffusion take place. Transepithelial electrical resistance (TER) is calculated from the slope of alternating adaptive DC voltages (U) and from its corresponding currents(I). The transepithelial potential difference (TEP) was measured using KCl electrodes. The short circuit current (SCC) was calculated as SCC = TEP/TER. The TER mainly reflects the ion permeability of the tissue and it is a proxy for barrier integrity. Whereas, the TEP measures the net ion distribution across the epithelium and SCC shows the net active ion transport.

## Methods

Rainbow trout skin explants were mounted in Ussing chambers at 10 °C. Explants acclimated for 3-4 h before standardized superficial wounding, which was performed directly in-chamber by scraping a section of the epidermis. The epidermal (apical) side was exposed to freshwater and the dermal (basolateral) side to physiological saline solution. TER, SCC and TEP were recorded repeatedly (5-min intervals; analyzed in 30-min bins). Repeated-measures one-way ANOVA with Dunnett adjustments compared post-wound values to pre-wound baseline (paired design; N = 24). To validate this approach, histological analysis was carried out.

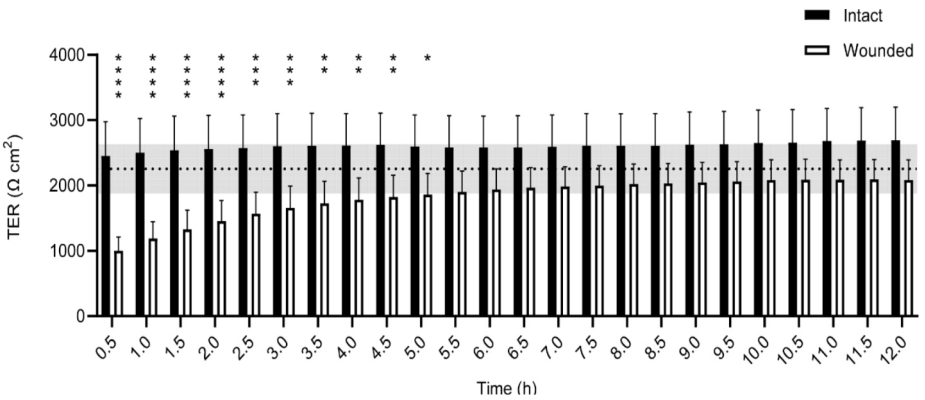


Fig 1 Transepithelial resistance (TER) remains stable for the intact skin and increases for the wounded skin. At 5.5h, there is no significant difference between the wounded and baseline TER.

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**Results & Discussion**

TER remains stable for the intact skin and increases for the wounded skin (Fig 1). At 5.5h, there is no significant difference between the wounded and baseline (dotted line and grey band) TER. This indicate that wounding caused an immediate TER drop followed by rapid recovery. TEP remains relatively stable for the intact skin but slightly trends towards zero over the course of the experiment. But, the wounded skin trends towards a higher, more negative TEP, closer to its baseline. SCC of the intact skin decreases slightly over the experiment but remains stable from around 4h. The wounded skin shows a high SCC immediately after wounding, but becomes consistent with the intact skin over the course of the experiment.

The TER increase in wounded skin relative to the control skin indicate that a wound healing response was occurring like cell migration. The histological assessment confirmed the presence of a neoepidermis and found that a neoepidermis was present within the first 12 h pw.

**Conclusions**

This study is a positive step towards a functioning *ex vivo* wound healing model. It provides high-resolution, real-time observations of the earliest phases of wound healing in fish and is well-suited for testing environmental or husbandry modifiers relevant to aquaculture. It offers a tissue-complex alternative to cell-only assays while supporting reduction and refinement principles.

# SECONDARY TREATMENT OF RAS WATER AND NUTRIENT REVALORIZATION BY SITE-SPECIFIC MICROBIAL BIOFILM

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**Introduction**

Land based aquaculture systems, such as Recirculating Aquaculture Systems (RAS), produce excess water highly rich in Nitrogen (N) which must be treated. The release of N-rich water in the environment can lead to undesirable eutrophication, and regulations, to avoid and control this, are increasing. In this context, the existing solution, besides releasing to close water bodies, is fixating the N back to air. These solutions are not ideal in terms of sustainable use of the nutrients and do not promote resource revalorization. At the same time the feed industry is looking for new and more sustainable ingredients to supplement their feed. Several systems focusing on the uptake and valorization of nutrient rich side stream have been proposed, including coupling land-based fish farms with plant growth in aquaponic systems or with bioreactors growing a microalgae monoculture.

**Materials and methods**

The water used for the experiments was collected in a representative Norwegian RAS installation during four separate samplings throughout a time period of 10 months. The experiments were performed either in culture flasks or reactors at 13°C with a constant light intensity of 203-333 μmol $m^{-2}$ $s^{-1}$. We tested how conditions such as harvesting rates, nutrient concentration and light affected the growth of the biofilm. We evaluated the bioremediation capacity and performed eurokaryotic and prokaryotic community analysis.

**Results**

We found that in this reactor, organisms naturally present in the RAS form a biofilm and can efficiently bio-remediate the water by reducing the $N\text{-}NH_3$ up to 76.6%, $N\text{-}NO_3^-$ up to 84.9% and the $P\text{-}PO_4^{3-}$ up to 95.7%. Sequencing results indicate that the eukaryotic community was, in all conditions, strongly dominated by the diatom *Phaeodactylum tricornutum,* almost resembling a monoculture.

# EVENT BASED FISH DETECTION IN A CYBER-ENHANCED TANK. TWO NOVEL CONCEPTS FOR PRECISION FISH FARMING

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## Introduction

Aquaculture has seen significant changes the recent years, since aquatic resources are considered a primary protein supplement for daily lives in many communities. The aquaculture industry is experiencing a steady increasing production, with an approximate average annual growth rate to 1.72 % since 2010 [1]. Common practices for fish farming require standard procedures that are considered expensive and time-consuming. In recent years, science has presented outstanding novelties, featuring a wide pallette of technological advancements. Frame- and event-based cameras, scanning sonars and light enrichment are some of this wide variety that can create novel methodologies for fish farming.

In our project, two new concepts are introduced: a cyber-enhanced tank and an innovative fish behavior detection via neuromorphic vision [2]. The event camera, which is the basis for the neuromorphic vision, can detect alterations in fish movements by analyzing the generated changes in pixel light intensities. We propose an experimental tank setup with these technological deployments which we believe will provide a more realistic environment for the fish and a multi-modal way of observing fish behavior via a set of (frame and event) cameras and a scanning sonar.

## Materials and Methods

### Cyber-enhanced tank

The enhanced tank was built upon an existing conventional tank environment of four cubic meters. A waterproof LED line, illuminating the volume homogeneously with a spectrum close to sunlight (5600K to 6500K), was placed a few centimeters below the surface on a specially designed support frame, providing light conditions that mimic those in a commercial cage-mimicking light conditions. As displayed in Figure 1, the lights (operated by a signal frequency driver) follow the inner perimeter of the tank. A surveillance camera (I), a stereo camera (II), an event camera (III) and a sonar (IV), consist a multi-observatory sensor suite.

### Neuromorphic vision

A novel event-based fish monitoring procedure is presented via neuromorphic vision, by detecting alterations in pixel brightness (known as events). This differs from the operation of commercial frame-based cameras. Figure 1-III shows an example of outputs from this device, where two highly mobile fish are detected.

The black and blue dots represent the variations in the light intensities of the pixels. The main goal of this procedure was to count the events generated by the fish during different stimuli presentation. As an example, Figure 2 shows feeding operations recorded by the same event camera from two different perspectives, bottom (left) and top (right) placement, respectively. The red dotted lines denote the feeding time while blue lines depict the event time series.

## Conclusions

This study introduces two new concepts for aquaculture research: a cyber-enhanced tank that provides a realistic tank environment and a sensor suite for fish monitoring, and a new method for behavioral monitoring based on neuromorphic cameras. The cage-like tank provides light conditions similar to real aquaculture settings, aiming to promote fish welfare during experiments. Neuromorphic vision represents an innovative technology for fish detection, a concept that can provide more information for industrial use.

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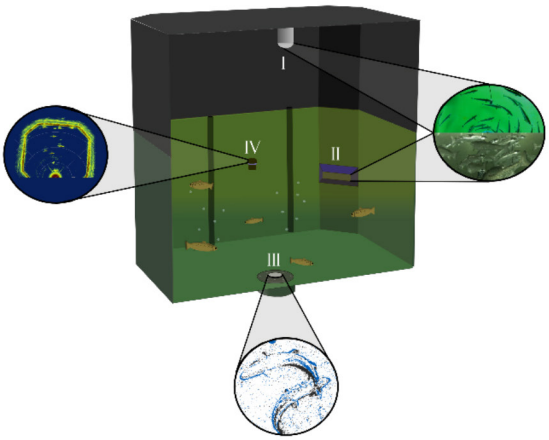


Figure 1. 3D experimental tank design.

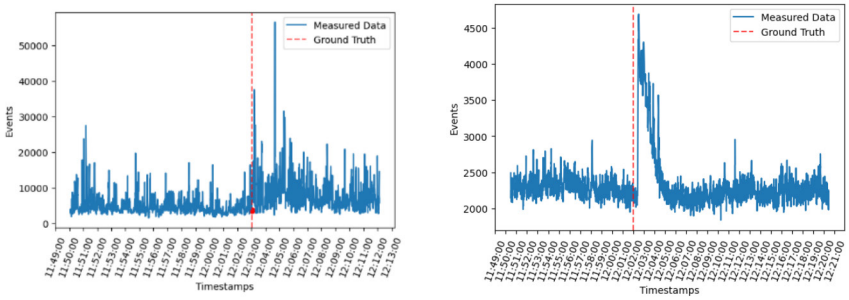


Figure 2. Fish behavior detection during feeding procedure (Left - Bottom deployment, Right - Top deployment).

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# PRESENT STATUS OF BIOFLOC TECHNOLOGY SHRIMP CULTURE SYSTEM IN SOUTHERN BRAZIL

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## Introduction

The shrimp culture in biofloc technology systems (BFT) is characterized by zero water exchange and super-intensive culture of shrimp in enclosed raceway type. This type of system is considered environmentally friendly and avoids nutrient rich waste from polluting coastal waters. However, as a new strategy for shrimp production, there is much still to be learned about the benefits of producing shrimp BFT systems to enhance the commercial potential of the system. For that reason, several experiments have been carried out trying to find some of the keys for technical and economic feasibility of BFT shrimp superintensive culture systems.

## Materials and methods

Over the past 20 years, numerous studies have been conducted at various experimental scales. Sets of 300-, 500-, and 1,000-L tanks were used to conduct more precise tests. For pilot and commercial-scale tests, 40-$m^3$ and 250-$m^3$ raceways were used in super-intensive systems located in greenhouses. For experiments in intensive systems, 600-$m^3$ lined ponds were used.

## Results and discussion

Technological innovation permits increases in shrimp production capacity per unit area. The addition of new management tools such as air injectors (nozzles), nitrification and denitrification process, use of clarifiers and some new procedures may allow increases in the load capacity of these systems.

For example, the demand for dissolved oxygen (DO) by shrimp is one of the main concerns as culture systems intensify. In intensive or super-intensive culture of shrimp can be possible if the system's aeration devices can meet the organism's oxygen demand. The number of devices to be used will depend on the water's salinity and temperature, stocking density, shrimp size, and the amount of suspended solids in the BFT systems. For this reason, studies and evaluations of the support capacity of each new aeration technology is important to maximize its effect.

Therefore, several researchers have evaluated the influence of the limitation of different parameters and procedures. These are important challenges to overcome production when working in BFT with high densities, and different devices and other procedures (Table 1), that can have significant impacts on shrimp development in super-intensive system.

## Conclusion

In the present study we will present evaluations of some of these important generated technologies for BFT systems, and we will discuss the influence of high stocking densities on water quality and on the growth performance of *P. vannamei* in a BFT system.

*(Continued on next page)*

Table 1: Recent developed technologies for Biofloc Technology super-intensive shrimp culture systems in Southern Brazil

| BASIC CARACTERISTICS OF BFT SYSTEMS |
|---|
| FACILITIES: SIZE, FORMAT, MATERIALS |
| THE USE OF MICROBIAL LOOP: BIOFLOC |
| NITRIFICATION PROCESS |
| HIGH PRODUCTION OF BACTERIAS |
| MANAGEMENT OF ALKALINITY/PH/CO2 |
| HIGH CONCENTRATION OF TSS |
| AERATION SYSTEMS |
| USE OF VERTICAL SUBSTRATES |
| WATER MANAGEMENT |
| HIGH STOCKING DENSITIES |
| FEEDING MANAGEMENT |
| USE OF PROBIOTICS |
| LOW OR ZERO WATER EXCHANGE |
| REUSE OF WATER |
| DENITRIFICATION PROCESS |
| OTHERS |

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# TURNING A MULTI-CRITERIA DECISION TOOL FOR AQUACULTURE SITE SUITABILITY INTO AN INTERACTIVE, PUBLICLY ACCESSIBLE GIS PLATFORM

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## Introduction

Implementing proactive and effective spatial planning in aquaculture requires users and stakeholders to access decision support tools (DSTs) that centralize information to support site planning (Falconer et al., 2020). Spatial DSTs in the forms of models (e.g. carrying capacity) and mapping tools, are increasingly recognized for their role in enhancing aquaculture planning and management (Stelzenmüller et al., 2017; Gangnery et al., 2021). This paper presents the Coastal Classification System (CCS), a new publicly accessible web-based decision-support tool developed to support aquaculture planning and siting in Nova Scotia, Canada. The tool presents the results of province-wide suitability assessments and provides users access to various GIS map layers relevant to four cultured species: Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss*), Blue mussels (*Mytilus edulis*), and American oyster (*Crassostrea virginica*).

## Methods

The Coastal Classification System was developed through collaboration between the Government of Nova Scotia and the Centre for Marine Applied Research. The goal of this project was to create an online mapping platform to support regional-level planning about aquaculture in Nova Scotia (**Fig. 1**). The DST was built through a stakeholder-engaged process involving government, scientists, and industry, who provided input both on inclusion of relevant data, and on the useability of the outputs. It was built with accessibility and transparency in mind, aiming to provide information about aquaculture to a range of users.

## Results

The CCS operates as an online platform using ArcGIS products. A web-based mapping tool (Fig.2) provides users access to over 20 spatial layers of information, including generated suitability maps and data layers relevant to assessing aquaculture suitability. Users can also overlay various informational data layers alongside the suitability maps. To enable area-specific investigations, users can zoom in to any area within the area of assessment and click on each 'cell' (100 m resolution) to view more information about how the area was assessed.

To support the mapping tool, an ArcGIS Story Map was also developed, providing additional information about the suitability assessment, each data layer, as well as guides on how to use the tool. The CCS is publicly available for anyone to access, view, and explore.

## Discussion

The developed online mapping platform not only increases transparency, provides information related to datasets and opportunities, but also supports future planning. With this tool, planners, decision-makers, and prospective producers can understand not only where it is potentially suitable for development, but also the types of information and datasets to consider in their site applications. The tool centralizes information and provides easy to understand language to evaluate where and why an area may have greater or fewer opportunities for development. Furthermore, this tool can help support future decisions around locations for subsequent strategic assessments, and also where there may be data gaps. This transparent, accessible platform was built as a living tool to allow further improvement and can be readily adapted to incorporate new data and meet the needs of the evolving industry.

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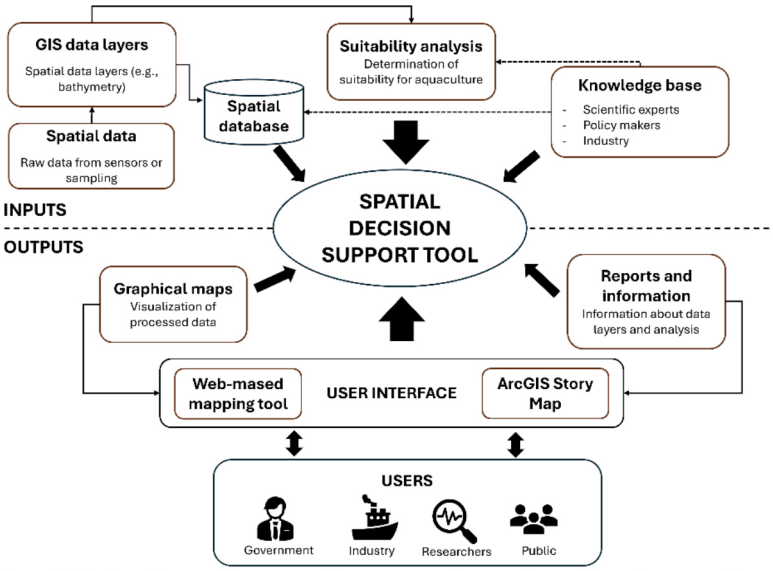


**Fig. 1.** The CCS decision-support tool integrates data and analysis inputs with accessible and transparent outputs.

**Results**

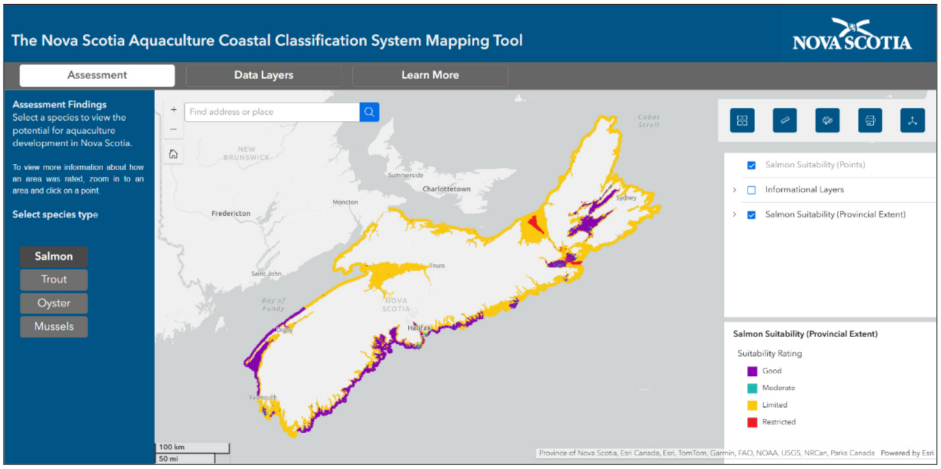


**Fig.2.** Screenshot of public mapping tool, where users can navigate results of suitability assessments and view various data layers relevant to aquaculture in Nova Scotia, Canada.

## References

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# WHERE TO LOOK? A PROVINCE-WIDE SUITABILITY ASSESSMENT OF MARINE FINFISH AND SHELLFISH AQUACULTURE IN NOVA SCOTIA, CANADA

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## Introduction

As aquaculture seeks to expand into new areas, responsible planning for favourable areas is needed to avoid conflict and promote sustainable development (Aguilar-Manjarrez et al., 2017). Identifying suitable areas for aquaculture requires comprehensive evaluations of multiple biophysical and ocean use factors. Many of these factors are inherently spatial, varying across areas and locations. As such, spatial modelling has become increasingly applied to inform site selection and broader area-based planning globally (Chentouf et al., 2023; Silverthorn et al., 2025). Here, we present the findings from a comprehensive suitability assessment for potential coastal aquaculture development in Nova Scotia, Canada. A province-wide decision-support tool was developed, called the Coastal Classification System (CCS).

## Methods

Through evidence-based and collaborative approaches, this project identified and assessed the various criteria that influence aquaculture suitability. Four marine aquaculture species were assessed: Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss*), Blue mussels (*Mytilus edulis*), and American oyster (*Crassostrea virginica*). Drawing on methods from Geographic Information Systems (GIS) and Multi-Criteria Decision Analysis (GIS-MCDA), suitability classifications were mapped within Nova Scotia's coastal waters (just over 13,000 km$^2$). A total of 15 criteria and constraints were selected to consider critical needs for species health and welfare, potential overlap with other marine users, and proximity to important coastal habitats. Criteria layers were scored based on parameter-specific suitability functions and aggregated using weighted linear combination, rule-based overlay, and constraint overlay.

## Results

Final suitability maps classify each area at a 100 m$^2$ resolution, as one of four ratings: Restricted, Limited, Moderate, or Good for potential aquaculture development. Across all assessed species, restricted areas accounted for only 2% of the area of assessment, predominantly in major traffic separation zones adjacent to high-use ports. For finfish species, roughly 3,500 km$^2$ (25-26%) of the area of assessment was classed as well-suited for potential aquaculture development, while this decreased to ~13.5% for shellfish species.

## Discussion

For different types of species, suitability mapping identifies unique areas for greatest sustainable aquaculture development potential.

The use of GIS-MCDA techniques to assess evaluation criteria and integrate data has allowed for the creation of practical outputs and maps for industry and decision-makers. This information supports early-stage planning to identify where opportunities for aquaculture development may occur and areas that may have limitations. This tool enhances proactive aquaculture planning and can be used to identify further areas for more local-scale investigations.

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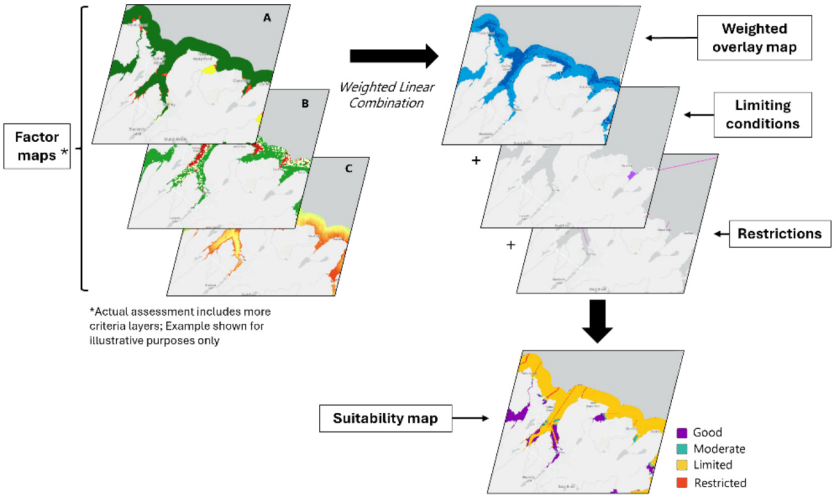


**Fig. 1**. Graphical representation of factor aggregation, restrictions and limiting conditions to produce an overall suitability map.

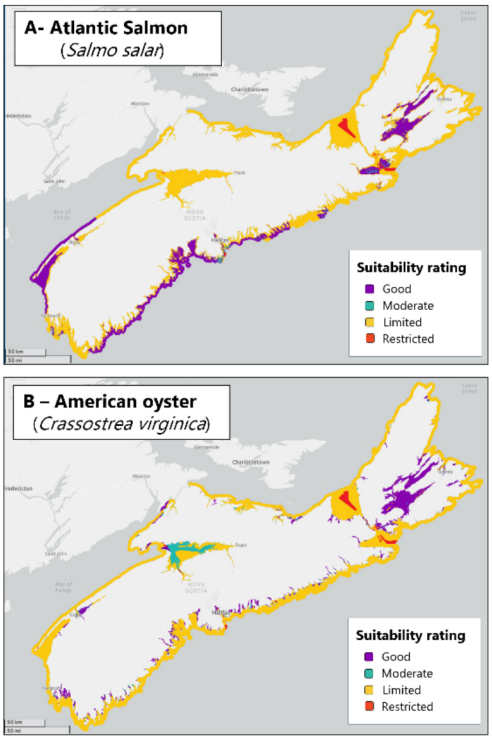


**Fig. 2**. Distribution of suitability ratings across the area of assessment for two assessed species.

# TURNING A MULTI-CRITERIA DECISION TOOL FOR AQUACULTURE SITE SUITABILITY INTO AN INTERACTIVE, PUBLICLY ACCESSIBLE GIS PLATFORM

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**Fig.2.** Screenshot of public mapping tool, where users can navigate results of suitability assessments and view various data layers relevant to aquaculture in Nova Scotia, Canada.

To support the mapping tool, an ArcGIS Story Map was also developed, providing additional information about the suitability assessment, each data layer, as well as guides on how to use the tool. The CCS is publicly available for anyone to access, view, and explore.

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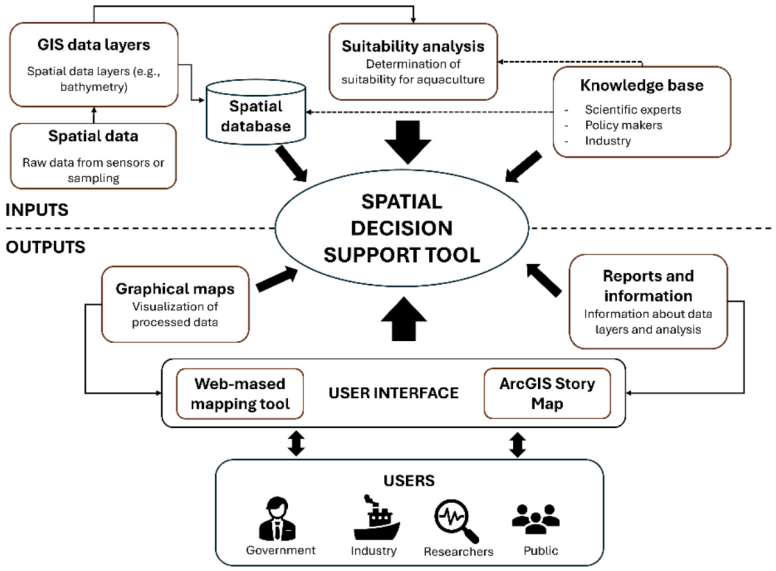
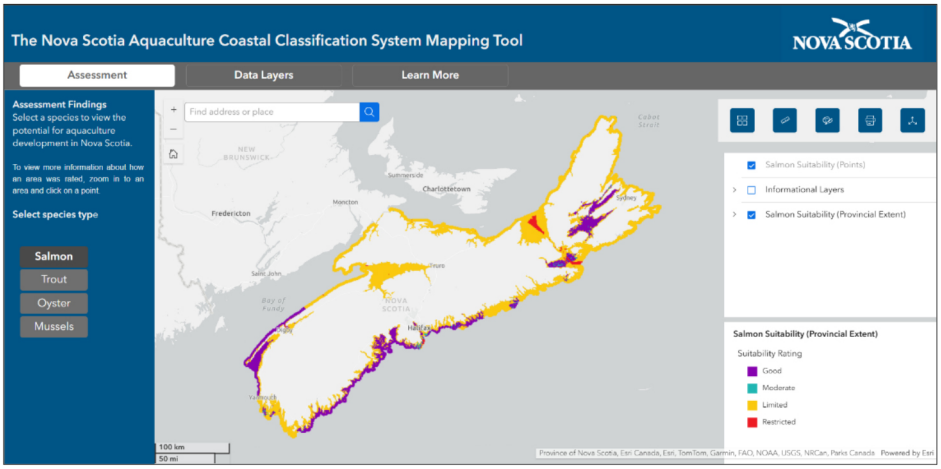



**Fig. 1.** The CCS decision-support tool integrates data and analysis inputs with accessible and transparent outputs.

**Results**

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# FROM SEA TO PLATE: ADVANCING SELECTIVE BREEDING FOR NON-SALMONID SPECIES TO DRIVE AQUACULTURE DIVERSIFICATION

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Diversifying aquaculture species is critical not only for building climate-resilient food systems but also for making more effective use of available water space for food production. In Aotearoa New Zealand, the Australasian snapper (Sparidae: *Chrysophrys auratus*) is emerging as a strong candidate for both coastal and open ocean aquaculture systems [1]. This promising warm-water species has close relatives—such as the red sea bream in Japan and the gilthead sea bream in the Mediterranean region—that are already well established in international aquaculture markets, underscoring its commercial potential. This talk presents recent genomic and phenotypic innovations underpinning the development of a selective breeding programme for this non-salmonid species

Over the past 20 years, snapper have been bred and trialled in both land-based and ocean-based systems, with selectively bred fifth-generation ($F_5$) fish showing marked improvements in growth, feed conversion ratio (FCR), and survival [2, 3]. Domestication selection [4], high-throughput phenotyping [5]—leveraging computer vision and machine learning—and genomics-based selection [6, 7, 8, 9, 10] have driven these production related gains in performance .

While early breeding efforts prioritised growth performance, the escalating effects of climate change have recently shifted the focus toward enhancing temperature resilience. Snapper are highly thermally sensitive [11]: growth is stimulated at temperatures above 15 °C, stalls below this threshold, and fish begin to lose condition as temperatures approach 10 °C. Optimal growth occurs near 21 °C, underscoring the influence of environmental temperature on aquaculture viability. In the wild, snapper populations in New Zealand are expanding southward into regions that were previously unsuitably cold, likely facilitated by ocean warming [12, 13, 14]. However, the persistence of low winter temperatures in these areas continues to limit further range expansion and poses significant challenges for survival and growth. In response, we have initiated New Zealand's largest temperature resilience trial (four temperature treatments, each with 4 replicated tanks, starting snapper numbers per tank 200), involving over 3,000 GBS-genotyped snapper reared under four distinct thermal regimes for 12 months. In addition to high-frequency (8-weekly) phenotyping, the trial integrates whole genome SNP data with transcriptomic and epigenomic profiling to uncover the molecular basis of thermal resilience and growth.

Launched in April 2025, this comprehensive study is producing critical data on genotype-by-environment interactions and physiological responses to thermal stress, with the goal of informing selective breeding strategies for climate-adapted aquaculture. I will present first results from this trial alongside data from recent grow-out comparisons between selectively bred and wild-type fish. Together, these findings highlight the potential of snapper as a climate-resilient aquaculture species and underscore the importance of integrating environmental robustness into breeding strategies for future-ready aquaculture systems.

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# RE-IMAGINING OPEN OCEAN AQUAQUACULTURE: DESIGNING WITH THE FISH IN MIND

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## Introduction

Global seafood demand is anticipated to increase over the long term, with finfish mariculture poised to become a major contributor to future supply [1]. As sheltered, nearshore marine environments are highly valued and regulated and are already occupied in many areas of the world, open ocean aquaculture (OOA) is the most likely way to meet global demand for finfish.

A typical approach to design OOA fish enclosures is to enlarge and fortify existing technologies with the aim to both increase production biomass and to withstand extreme oceanographic forces [2,3]. The New Zealand-based Whakapōhewa ki ahumoana Reimagining Aquaculture project (funded by the Ministry for Business, Innovation and Employment Endeavour Fund) lead by Plant & Food Research, has taken a different approach. Focussing firmly on the needs of the fish, they have designed a system to support healthy fish growth, limit adverse impacts on the marine environment and withstand the physical impacts of sometimes harsh and unpredictable offshore conditions [4].

This presentation describes the very first biological trials of this soft-engineered, semi-mobile aquaculture production system with two exemplar species: Australasian snapper/tāmure/silver sea bream (*Chrysophrys auratus*) and Chinook/King salmon/hāmana (*Oncorhynchus tshawytscha*).

## The innovation: a semi-mobile aquaculture production system

A 500m$^3$ soft-engineered fish enclosure (20m x 5m x 5m) was constructed utilising a laminated ultra-high molecular weight polyurethane fabric with nets placed at the front and back of the enclosure (Figure 1).

The fish enclosure is tethered to a mooring setup allowing the system to swing about the mooring as the tide changed. A support and monitoring buoy (Figure 1b and 2) delivers feed as well as providing a conduit for sensors and underwater camera cabling for monitoring fish inside the enclosure via a flexible umbilical tube to a depth of 15 m.

In early April 2025, approx. 2000 snapper were transported to the trial site and introduced to the system (Figure 2) where they resided in the system for 6 weeks, with approx. 3000 salmon transferred in July. Both species were fed an appropriate commercial pellet diet. Remote monitoring of fish was possible through a continual live underwater video feed.

## Results

Fish were successfully cultured in the semi-mobile system, gaining weight and maintaining health and condition during the trials. The system and fish were subject to a number of substantial weather events during the trial period. Being submerged to a depth of 15 m meant that significant wave motion was avoided compared with surface waters. Feed, monitoring and remote communications were maintained throughout the trial.

## Discussion

To our knowledge, this is the first large-scale demonstration of finfish successfully reared in a submerged, soft-engineered, and semi-mobile aquaculture system. The integration of underwater feeding and continuous video monitoring enabled deployment at a high-energy offshore site and facilitated trials across two biologically distinct species. Engineered around fish welfare, the system can be fully mobile—capable of being towed to track favourable thermal and water quality conditions. This novel, fish-centred approach represents a step-change in offshore aquaculture, offering a low-impact, scalable solution with the potential to reduce capital and operational costs while unlocking under-utilised open ocean areas for sustainable finfish production.

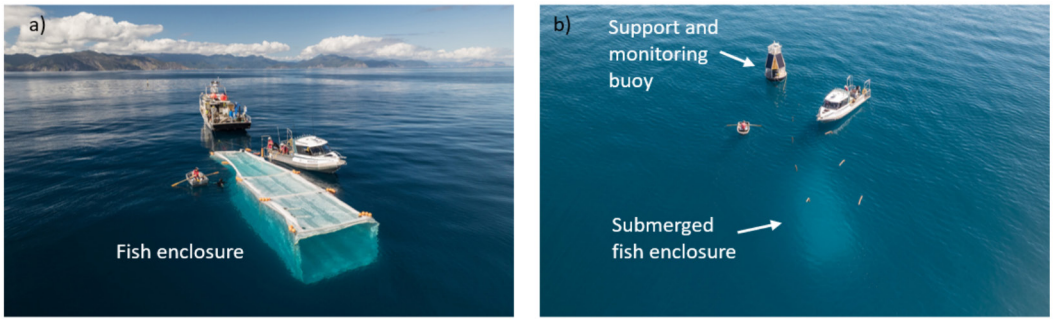


Figure 1. Fish enclosure at the surface (a), and submerged to an operational depth of 15 m (b).



Figure 2. Snapper inside the fish enclosure.

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# INVESTIGATING ABIOTIC AND BIOTIC DRIVERS OF GROWTH AND REPRODUCTION IN ROPE-CULTURED MUSSELS *Mytilus* spp. IN AN IRISH (TEMPERATE) AND NORWEGIAN (SUBARCTIC) FISHERY.

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**Introduction**

Mussel aquaculture across Europe is dominated by blue mussels *Mytilus edulis* in the north and Mediterranean mussels *Mytilus galloprovincialis* in the south, with hybridization occurring where ranges overlap. *M. galloprovincialis* expanded into Ireland in the 1970s (Gosling & Wilkins, 1977), and hybrid zones are now common on exposed Irish coasts (Lynch et al., 2020). In Norway, *M. edulis* has been the predominant species, but recent studies report patchy distributions including *M. trossulus* and *M. galloprovincialis* (Simon et al., 2020). Wild and commercial *Mytilus* populations have declined across parts of the North Atlantic due to warming seas, predation, disease, and habitat disturbance (Baden et al., 2021). Ireland produces >10,000 t of rope-grown mussels annually (MSC-certified, ~€14M value), whereas Norway's production remains relatively small (~2,600 t in 2022) (BIM, 2024; Bruvold et al., 2024). Challenges common to both industries include inconsistent growth, poor spawning synchrony, and unreliable spat settlement (Baden et al., 2021; Mortensen et al., 2023). Growth performance directly affects mussel aquaculture profitability and harvest timing, with market size typically 20–25 g per mussel (FAO, 2023) while pathogens/parasites (e.g., *Vibrio* spp.) remain a monitoring concern, with variable prevalence by site and season (Díaz et al., 2022).

This study aimed to characterise and compare age performance, whole wet weight (WWW), species composition, sex ratio, spawning, and *Vibrio* spp. prevalence of rope-cultured mussels in a temperate system Roaringwater Bay (Ireland) and a subarctic system Rundhaugen (Norway). This comparison provided a unique opportunity to investigate how contrasting climatic regimes influence mussel growth, reproduction, and disease dynamics. While both regions employ longline aquaculture, they differ substantially in thermal regime, photoperiod, and primary productivity cycles. Key environmental drivers (sea surface temperature (SST) and chlorophyll-*a* concentration) were evaluated. The findings of this study provide an evidence base for optimising rope deployment timing and improving site-specific grow-out management strategies.

**Methodology**

Seasonal quarterly sampling (April 2023–June 2024 (preharvest)) resulted in the collection of 600 mussels per site (1+ and 2+ year cohorts) at Roaringwater Bay, Ireland (51.526° N, 9.436° W) and Rundhaugen, Norway (65.13428° N, 12.32661° E), providing a latitudinal difference of 17.5° (Figure 1). Whole wet weight (WWW) and shell length were recorded. Gill tissue was sampled for DNA extraction (Chelex-100 method) and species identification via PCR (Me15/Me16 markers, Inoue et al., 1995): *M. edulis* 180 bp, *M. trossulus* 168 bp, *M. galloprovincialis* 126 bp, hybrids = dual amplicons). Gonadal histology determined sex and gametogenic stage. Sea surface temperature (SST) and chlorophyll-a were obtained from Copernicus and BIM datasets. A multiplex PCR was conducted to identify four specific *Vibrio* spp., as well as the general presence of *Vibrio* spp. (O' Leary et al., *In Revision*). Detection of the generic *Vibrio* spp. (419 bp), *V. aestuarianus* (259 bp), *V. spendidus* (182 bp) and *V. anguillarum* (519 bp) were recorded using gel electrophoresis. Regression Analysis was run to examine relationships between independent variables and a dependent variable. Pearson correlation coefficients were used to measure the strength and direction of a linear relationship between two continuous variables. Fisher's Exact Test was used to investigate significant relationships between categorical variables.

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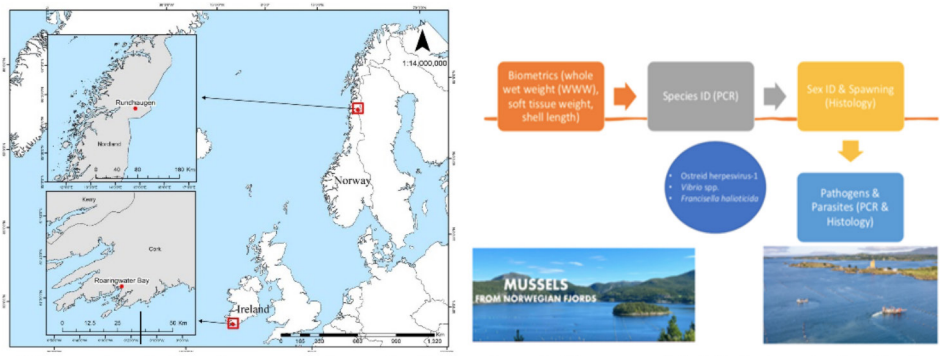


Figure 1 : Mussel rope fishery in Ireland and Norway, highlighting latitudinal difference and an overview of the methodology used.

**Results**

*M. edulis* dominated at both sites (77.7% in Ireland; >70% in Norway). *M. galloprovincialis* occurred at lower levels (≈10% Ireland, 23.8% Norway), and hybrids were only detected in Ireland. No *M. trossulus* were detected in both fisheries. Growth patterns differed significantly ($p<0.05$): Overall Irish mussels exhibited rapid weight gain to the end of the trial (June'24) in the Year1+ cohort (mean WWW 6.19 ±3 g) that slowed in the Year2+ cohort (mean WWW 10 ±4g). Norwegian mussels displayed steadier growth to the end of the trial in the Year 1+ cohort (mean WWW 5.5 ±2.5g) and Year 2+ cohort (mean WWW 11 ±3.5g). Size variability increased with age, particularly in Irish 2+ year mussels (spread ≈13.1 g in July'23).

Sex ratios were female biased and similar at both sites (Roaringwater Bay: 0.59:1-0.88:1; Rundhaugen: 0.53:1-0.88:1). Spawning occurred in both fisheries in the smaller sized/lighter (<14.9g) cohort in all sample months (all seasons) and in heavier mussels (>15g) in April'23 and July'23 in the Irish fishery and in April'23, October'23 and January'24 in the Norwegian fishery. Overall in Roaringwater Bay, males averaged a weight of 6.3 g (4.4 - 10.5 g), while females were lighter at 5.8 g ( 5.1 – 9 g); *M. edulis*, *M. galloprovincialis*, and hybrids showed distinct mean sizes, with hybrids intermediate, and spawning mussels were smaller than non-spawning.*M. edulis* was the heaviest species in both fisheries, consistently exceeding the weights of *M. galloprovincialis* and hybrids. Overall in Rundhaugen, males had a mean weight of 6.5 g (5.3 - 12.2g) compared to females at 7.2 g ( 4.9-13 g), with similar species-specific patterns and reduced size in spawning individuals.

Overall, minimal detection of *Vibrio* spp. was observed in Roaringwater Bay mussels (4.5%) and Rundhaugen mussels (2.8%). At Roaringwater Bay, moderate SST and reduced salinity in April'23 favoured a phytoplankton bloom, followed by a decline in chlorophyll-*a* from July'23 onwards. At Rundhaugen, rising SSTs and increasing chlorophyll-*a* from spring to summer supported gametogenesis, spawning, and growth, while moderate autumn conditions facilitated a second spawning event in the mussels in January'24.

**Discussion**

Hybrids were present in Ireland but absent in Norway suggesting reduced sympatry and potential selection against hybrids under colder conditions. Growth trajectories suggested different energy allocation strategies: Irish mussels grew rapidly in Year 1, then plateaued but this may also have been due to the relocation of the mussels in Year 2 higher up in the bay. Norwegian mussels grew more consistently, reaching higher final weights in the older cohort. Seasonal weight peaked in Norway during winter suggesting that growth is influenced not only by SST but also by reproduction-related energy trade-offs. Females dominated both fisheries and synchronised spawning occurred between the sexes in both fisheries. Spawning occurred in all sample months for the smaller/lighter mussels, while heavier individuals spawned in more defined energetically favourable time periods (spring/summer). These results would indicate that lighter mussels may lack energy reserves and spawn opportunistically whenever energy is available. It may also indicate that smaller mussels are more sensitive to environmental triggers (SST fluctuations, food pulses) and spawn irregularly. Irish mussels displayed one major spawning event (July'23), tightly coupled to peak SST (15 ºC) and the post-spring phytoplankton bloom, suggesting a build-up of energy reserves before releasing gametes. Spawning peaked twice in the Norwegian system, in July'23 which coincided with peak SST and chlorophyll-a and in January'24 after a secondary autumn bloom, when the SST was at its lowest but still within tolerance for spawning in cold-adapted *Mytilus* spp. Although a high energy trade-off is required by female mussels to produce larger and more nutrient rich eggs compared to the male sperm, females may benefit from spreading reproductive effort over time in both systems by avoiding the risk of "putting all eggs in one basket" if a spawning season fails.

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**Conclusion**

This first comparative analysis of rope-cultured mussels from Ireland and Norway highlights that both biotic (species composition, age, reproduction) and abiotic (SST, chlorophyll-a) factors jointly shape growth and reproduction. Monitoring SST trends and spring chlorophyll-a blooms can help predict the optimal deployment window in a given year. Findings support site-specific optimisation of rope deployment and harvest schedules to maximise settlement success and production yields, as well as informing that the movement of ropes mid grow out cycle to a new location may not always be beneficial.

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# ENHANCING SHRIMP COLOUR AND YIELD: EVALUATING THE IMPACT OF NATURAL ASTAXANTHIN IN PLANKTON SYSTEMS FOR COMPETITIVE FARMING IN THE EU MARKET

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## Introduction

The European shrimp market is renowned for its stringent standards regarding product quality, safety, and sustainability within the aquaculture industry. In this context, our study explores how the incorporation of astaxanthin into feed formulations—specifically through a mixed application at the feed mill—can enhance these critical attributes. Shrimp colour serves as a key sensory factor that signals product quality, playing a pivotal role in gaining access to the EU market. However, beyond aesthetic appeal, it is imperative for farmers to understand the overall impact and return on investment (ROI) of this nutritional enhancement to remain competitive against existing suppliers. Conducted on a commercial farm in Bangka Island, Indonesia, this study evaluates 13 out of 57 ponds and assesses various parameters, including coloration, meat quality, yield performance, and overall shrimp health—each of which directly influences profitability. Ultimately, the goal of this research is to translate scientific insights into commercially viable practices that meet the rigorous standards of the European market.

## Material and Method

All experimental ponds adhered to standardized procedures for pond and water preparation to ensure uniform conditions throughout the study. Each pond utilized the same high-quality commercial feed brand and specifications.

From Day of Culture (DOC) 30 to DOC 101, the experimental design involved administering astaxanthin-enriched feed exclusively to Treatment 2 and Treatment 3 ponds, while the control ponds continued to receive standard feed. Comprehensive data collection was implemented as follows:

1. Growth Performance Metrics: Weekly sampling incorporated parameters such as Mean Body Weight (MBW), Average Daily Gain (ADG), Survival Rate (SR), and Feed Conversion Ratio (FCR). Partial harvest assessments were conducted to gather these metrics systematically.

2. Coloration Assessment: At DOC 101, shrimp coloration was evaluated using a salmon fan and shrimp ruler from DSM. The assessment included samples of raw Highly Unsaturated Fatty Acids Oil (HOSO), cooked HOSO, and peeled and cooked thawed HOSO to determine the impact on pigmentation.

3. Post-Harvest Meat Quality: Meat quality analysis was performed by measuring drip loss and thawed loss. Shrimp specimens were frozen for a period of 24 hours at a temperature range of -1 to 0 °C. Drip loss was quantified after a 15-minute exposure to ambient conditions, followed by thawed loss measurements after an additional 2-hour period.

Experimental Setup

| No | Pond Name | | | Post Larvae | | Treatments | Antioxidant Feed Additives | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | Size | Density | Hatchery | Genetic | | Remarks | Type | Sources | Inclusion Rate |
| 1 | D2 | 2.086,13 | 180 pcs/m2 | CPP/BLK | Balance | T1 | No Astaxanthin | | | |
| 2 | D7 | 2.086,13 | | | | | | | | |
| 3 | D8 | 2.086,13 | | | | | | | | |
| 4 | D10 | 2.086,13 | | | | | | | | |
| 5 | D12 | 2.086,13 | | | | | | | | |
| 6 | E1 | 2.202,38 | | | | T2 | Aquacel | Natural | Bacteria | 3kg/MT Feed |
| 7 | E2 | 2.202,38 | | | | | | | | |
| 8 | E3 | 2.202,38 | | | | | | | | |
| 9 | E4 | 2.191,00 | | | | T3 | Yeast CP | Natural | Yeast / Basiomycetous | 1kg/Mt Feed |
| 10 | E5 | 2.191,00 | | | | | | | | |
| 11 | E6 | 2.896,13 | | | | | | | | |

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4. Biweekly Shrimp Health Parameters: Health evaluations included assessing aggregate total microvilli (ATM), tubular shape and content, health shrimp index, porous shrimp shell condition, chromatophore integrity, white muscle quality, melanisation, gill coloration, soft shell prevalence, hepatopancreas coloration, the occurrence of white spots, and measurements of body colour and shape, lipid size, and bacterial counts.

5. Biweekly Immunological Assessments:Immunological parameters were quantitatively analysed, focusing on lysozyme activity, peroxidase and superoxide dismutase activity, glutathione levels, glycogen content, and total haemocyte count to evaluate the immune responses of the shrimp.

6. Daily Water Quality Monitoring: Water quality metrics were meticulously recorded, including temperature, salinity, pH, dissolved oxygen (DO) levels, plankton species composition, and quantifications of ammonia, nitrate, and nitrite concentrations to ensure optimal culture conditions.

7. Yield Performance Evaluation: The yield performance was assessed at the final harvest to determine the overall efficacy of the treatment conditions.

8. Statistical Analysis: Data were subjected to descriptive statistical analysis, followed by Analysis of Variance (ANOVA), post hoc testing, and regression analysis, with significance established at $p < 0.05$.

This multifaceted approach provides critical insights into the impact of astaxanthin-enriched feed on growth performance, health status, immune function, and overall yield in shrimp aquaculture. Such findings contribute to a comprehensive understanding of the potential benefits of astaxanthin supplementation within aquaculture systems, paving the way for improved practices and enhanced product quality.

**Expected Outcome**

In this study, we present comprehensive data on optimal management practices aimed at enhancing shrimp coloration, meat quality, health, and yield performance. Our findings provide valuable insights into the effects of various feed additives on shrimp health and overall performance, offering actionable knowledge specifically tailored for local farmers seeking to optimize their aquaculture operations. Additionally, we shed light on the interplay between plankton management and feed additives, elucidating their collective influence on shrimp microbiomes and quality traits, which is critical for developing improved farming methodologies. Furthermore, we propose recommendations for optimal inclusion rates of feed additives, balancing financial performance with key shrimp quality metrics, thereby fostering sustainable practices within the industry. Collectively, these insights contribute to a holistic understanding of shrimp aquaculture, promoting enhanced productivity and sustainability.

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# NAKED CLAMS: A COMPREHENSIVE ANALYSIS OF THEIR GLOBAL POTENTIAL FOR COMMERCIAL AQUACULTURE

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## Introduction

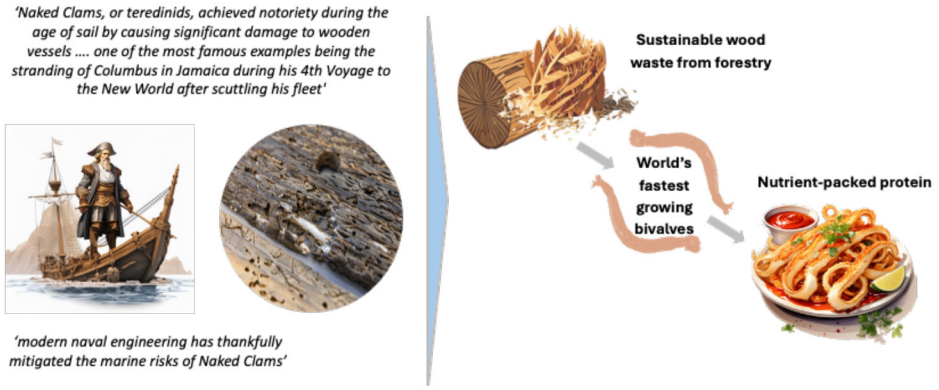
Naked clam aquaculture presents a game-changing solution for sustainable seafood production, capable of transforming waste wood into highly nutritious, low-environmental-impact protein for global consumption. Our previous articles in *npj Sustainable Agriculture* and *Food Frontiers* set the stage for naked clams. We demonstrated the world's first naked clam aquaculture system, nutritional analysis and global social media survey on their perception worldwide. This research showcased the groundbreaking potential of naked clams to turn waste wood into highly nutritious, low environmental impact protein that could feed millions of people worldwide, and captured international media attention, including coverage in the BBC, Financial Times, ITV, Sky News, and The Times, in addition to several aquaculture specific media outlets.

## Approach and Results

Our work presented today is the first comprehensive global analysis of naked clam aquaculture. By synthesising distribution, growth rate and size data across 24 species, we demonstrate that naked clams grow up to 3× faster than commercially farmed blue mussels, with some species exceeding 1.5 meters in length—despite the fact that their aquaculture has yet to be scientifically optimised. Large and fast-growing species are distributed globally, offering a readily available, scalable solution to sustainable seafood production. We further consolidate existing reports on their nutritional attributes, including their high protein content, vitamin B12 levels, and potential health benefits, while identifying critical knowledge gaps requiring further research.

To support the development of a scalable, low-impact aquaculture sector, we outline a strategic roadmap addressing species selection, growth optimisation, and disease prevention, offering a clear framework for future research and commercialisation. This work not only delivers new biological insights but also positions naked clams as a high-yield, low-carbon alternative to conventional aquaculture species, offering a transformative opportunity for sustainable seafood production while fully integrating into circular economies by converting waste wood into nutrient-dense protein, thereby closing resource loops and valorizing forestry byproducts.

The team our currently working on scale-up of naked clams within an IMTA system.



# USE OF CORN FERMENTED PROTEIN AS AN ALTERNATIVE SUPER PROTEIN IN DIETS FOR A RANGE OF COMMERCIAL AQUCULTURE SPECIES

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**Introduction**

Feed represents approximately 80% of the environmental footprint of aquaculture farming. Europe relies heavily on imported proteins to supply over 70% of the protein required for feed formulation for livestock and aquaculture (the Protein Gap). Soybean meal forms the major proportion of this imported protein meal and the challenge for the European feed industry is to source a locally produced protein meal that can replace soybean meal in ration formulation. Corn Fermented Protein (CFP) is a Super (>50% crude protein content) corn protein product which is produced by mechanical separation of the whole stillage fraction from the dry grind production process used to produce bioethanol a renewable transport fuel that reduces GHG emissions by 72% and fosters rural development. Produced as a co-product in the production of a renewable fuel, the carbon index of CFP is approximately one tenth of the production of an equivalent soybean meal protein product. During the 50-60h fermentation of starch to produce ethanol a large quantity of yeast is generated, approximately 8% of the grain starch is used in the generation of yeast. The yeast generated in the process passes through distillation and in the whole stillage remains as yeast cell wall material. Corn fermented protein contains 25% yeast cell wall material of which 44% is protein in the dry matter, derived from a fermentation process. The yeast cell mannans and glucans when used in the rearing period help improve innate and adaptive immunity. CFP is an alternative protein with bioactive components. There are many claims for the benefits of fermentation in terms of improved digestibility and mineral availability of plant material. The feed industry requires concrete evidence of efficacy before it adopts a new product. Over 50 published trials have been completed to demonstrate how to formulate feeds using CFP as a replacement of a wide range of currently used protein supplements in diets for all classes of livestock and companion animals. A major part of this program has been to evaluate the use of CFP in aquaculture species and this presentation summarises the data generated in these trials.

**Results**

Digestibility trials (stripping collection methodology) were carried out with Rainbow Trout (*Oncorhynchus mykiss*), average weight 250g. Average of 5 separate trials. Ingredient apparent digestibility coefficients of each nutrient in the test diet and ingredients were calculated using the traditional formulas presented in Kleiber (1961) and Forster (1999) (Table1).

The digestibility of the protein is approximately 88% measured in salmon and trout. Growth trials have been completed with salmon, trout, tilapia, shrimp and juvenile channel catfish summarised in Table 2.

**Discussion and Conclusions**

The data indicates that CFP is an excellent alternative protein and can replace a range of currently employed protein materials for use in diets for a range of commercially important aquaculture species. There are three unique characteristics of the product that merit note. Corn fermented protein is the product of a 50-60h fermentation process which increases the digestibility of protein and availability of phosphorus. The yeast component of CFP provides additional health benefits and since the product is a co-product of the generation of renewable fuel, CFP has a uniquely low Carbon Index valuable in European formulations.

*(Continued on next page)*

Table 1 Apparent digestibility coefficients of CFP in trout

| | Dry matter % | Protein % | Fat % | Energy % | Sulfur % | Phosphorus % |
|---|---|---|---|---|---|---|
| CFP 50 | 71 | 86 | 92 | 73 | 71 | 96 |
| CFP 60 | 73 | 89 | 93 | 79 | 67 | 73 |

*(Continued on next page)*

**Table 2. Summary of aquaculture trials performed using CFP to replace combinations of animal protein and soybean meal and soybean meal concentrates***

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Salmon 1: CFP% inclusion[a]** | | **0** | **5** | **10** | **15** | **20** | | **P value** | |
| | WG (g) | 425 | 408 | 446 | 385 | 358 | | 0.04 | |
| | TGC | 0.196 | 0.185 | 0.2 | 0.179 | 0.169 | | 0.01 | |
| | FCR | 0.98 | 0.93 | 0.93 | 0.97 | 0.97 | | 0.28 | NS |
| **Salmon 2: CFP% inclusion[b]** | | **0** | **7.5** | **10** | **15** | | | | |
| | WG (g) | 692 | 681 | 649 | 651 | | | 0.47 | NS |
| | TGC | 0.25 | 0.247 | 0.24 | 0.24 | | | 0.45 | NS |
| | FCR | 1.04 | 1.04 | 1.04 | 1.07 | | | 0.36 | NS |
| **Trout 1: CFP% inclusion[c]** | | **0** | **6** | **12** | **18** | **24** | | | |
| | WG (g) | 212 | 216 | 215 | 224 | 216 | | <0.05 | |
| | SGR % | 3.2 | 3.2 | 3.21 | 3.24 | 3.21 | | | NS |
| | FCR | 0.89 | 0.9 | 0.91 | 0.87 | 0.97 | | | NS |
| **Trout 2: CFP% inclusion[d]** | | **Commercial control** | **Corn Gluten meal 18%** | **Protein concentrate 18%** | **CFP18%** | | | | |
| | WG% | 626.7 | 676.1 | 578.8 | 701.7 | | | | |
| | SGR % | 2.83 | 2.94 | 2.74 | 2.99 | | | | |
| **Tilapia: CFP% inclusion[e]** | | **0** | | **10** | | **20** | **30** | | |
| | WG (g) | 633 | | 637 | | 635 | 569 | | |
| | FCR | 1.2 | | 1.18 | | 1.19 | 1.22 | | NS |
| **Shrimp 1: CFP% inclusion[f]** | | **0** | | **10** | | **20** | **30** | | |
| | Final biomass | 225.8 | | 204.6 | | 191.4 | 199 | | NS |
| | FCR | 1.61 | | 1.72 | | 2.05 | 2.12 | <0.05 | |
| | Survival | 76.7 | | 73.3 | | 80 | 85.8 | | |
| **Shrimp 2: CFP% inclusion[g]** | | **0** | **6** | **12** | **18** | **24** | | | |
| | Final biomass | 41.9 | 46.8 | 46.2 | 41.5 | 37.6 | | | |
| | FCR | 1.81 | 1.67 | 1.74 | 1.94 | 2.1 | | | |
| | Survival | 82.5 | 87.5 | 90 | 90 | 87.5 | | | |
| **Shrimp 3: CFP% inclusion[h]** | | **0** | | | | **20** | **30** | | |
| | Final biomass | 12.91 | | | | 13.43 | 15.4 | | NS |
| | FCR | 3.07 | | | | 3.16 | 3.1 | | NS |
| | Survival | 70 | | | | 82 | 84 | | NS |
| **Channel catfish CFP% inclusion[i]** | | 0 | 25 | 50 | 75 | 100 | | | |
| | WG (g) | 43.2 | 42.9 | 42.2 | 39.7 | 37.9 | | <0.009 | |
| | FCR | 0.9 | 0.9 | 0.9 | 0.87 | 0.79 | | <0.02 | |
| | Survival (%) | 78 | 82 | 83 | 89 | 88 | | <0.078 | |

*For precise details of the trials included in this table please please refer to the senior author

[a]40% animal protein: and replacement of soy protein concentrate

[b]15% fishmeal: replacement of protein concentrates

[c]21% fishmeal: replacement of soy protein concentrate

[d]18% inclusion comparison of protein concentrates

[e]5% fishmeal: replacement of soy bean meal

[f]Total replacement of 11% fish meal plus partial replacement of soybean meal

[g]Total replacement of 12% fish meal plus partial replacement of soybean meal

[h]6% fish meal: replacement of soybean meal

[i]100% replacement of soybean meal; bacterial challenge with virulent strain of *Edwardsiella ictaluri*

# EDU-FISH - FISHERY EDUCATION AND PROMOTION PROGRAM IN POLAND

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**Introduction**

A project called "Edu-fish fishery education and promotion program" acronym EDU-FISH has been implemented in Poland since October 2024 under Priority II of the European Maritime, Fisheries and Aquaculture Fund (FEDR) for 2021-2027. Project value: 23,910,692.96 PLN, including EU funding 16,661,036.05 PLN. The program was established through close cooperation of people concerned about the shortage of human resources in the fishing industry and the low number of candidates for fishery studies. A consortium of 3 fishery education institutions in Poland was formed in 2024: West Pomeranian University of Technology in Szczecin (ZUT) - Leader, the University of Warmia and Mazury in Olsztyn (UWM) and the School Complex in Sieraków (ZS), which prepared the EDU-FISH project.

**Material and Methods**

The main purpose of the EDU-FISH Program is to increase human resources potential in fisheries by preparing qualified specialists in fisheries, ichthyology and aquaculture. The stated goal will be achieved as a result of a large-scale promotional campaign to disseminate information on educational opportunities in the ichthyology profession and on professional qualifications obtained at the secondary level, enabling one to obtain the professional title of inland fisheries technician, and at the academic level, enabling one to obtain the professional degree of engineer or master. The proposed EDU-FISH program includes also material assistance in the form of funding for scholarships and wages for apprenticeships, purchase of computers and field clothes for students, the renovation of laboratory and teaching rooms, and the purchase of teaching apparatus and equipment.

**Results**

As part of the implementation of the program, more than 60 promotional activities were carried out, e.g.: participation in educational and industry events, for example: the Fishing and Survival Fair in Kielce 2024, the Fishing Fair Rybomania 2025 in Poznan, the Educational Fair Perspectives 2025 in Warsaw. As part of the promotional campaigns, many workshops and promotional demonstrations at the UWM Center for Aquaculture and Ecological Engineering in Olsztyn were also held for high school and elementary school students. The ichthyological education was also promoted during visits to high schools, including, the Forestry Technical School in Tuchola, the Complex of Schools of Environmental Formation and Agribusiness in Giżycko, the Complex of Schools of the Agricultural Training Center in Radom, and others. The promotion of the ichthyologist profession and education in the field of ichthyology and aquaculture consisted of sharing advertising leaflets and promotional gadgets, giving interviews and sharing educational and industry experiences. Another promotional elements are the promotional campaigns using social media, such as: Facebook, Instagram, Tik-tok, YouTube. Our activities enjoyed great interest, especially among people passionate about fish, angling or aquatics. The activities planned in the EDU-FISH program will make it possible to prepare a qualified workforce ready to achieve economic and social goals tailored to changing administrative and climatic conditions.

Funded by the European Maritime, Fisheries and Aquaculture Fund (FEDR) for 2021-2027" No: FEDR.02.01-IP.01-0001/24.

# OPTIMIZING GROWTH PERFORMANCE AND GUT MICROBIOME DIVERSITY IN JUVENILE ASIAN SEA BASS (*Lates calcarifer*) WITH DIETARY SUPPLEMENTATION OF MANNAN OLIGOSACCHARIDES, *Yucca schidigera* EXTRACT, AND SOLID-STATE FERMENTED *Aspergillus niger*

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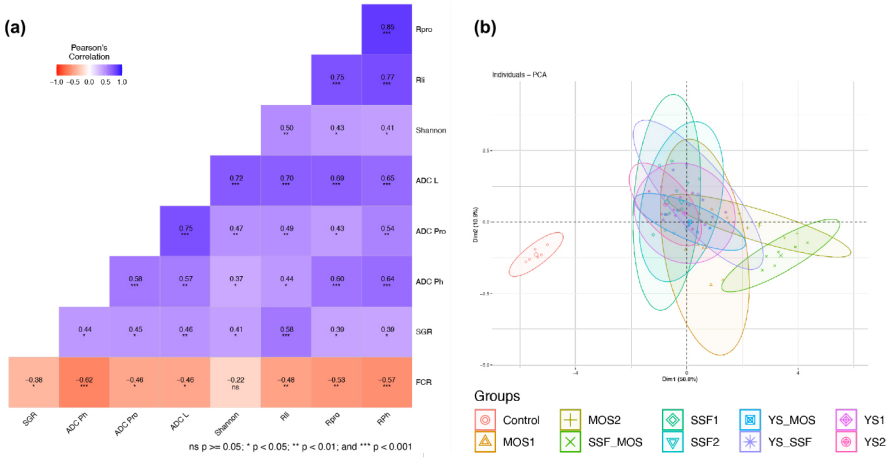
Asian seabass (*Lates calcarifer*), a commercial marine species in Southeast Asia, is prized for its quick development and adaptability, but it is susceptible to various diseases, particularly in dense environments. This study investigated growth performance and gut microbiome diversity using 16S rRNA metagenomic analysis in juvenile Asian seabass supplemented with Mannan Oligosaccharides (MOS), *Yucca Schidigera* extract (YS), and Solid-State Fermented Aspergillus niger (SSF) to optimize aquaculture practices. Fish were fed commercial diets supplemented with ten additive compositions for 70 days, assessing growth performance, feed utilization, and survival. The YS_SSF group showed superior weight gain (WG = 36.05 ± 1.10) and specific growth rate (SGR = 1.36 ± 0.03), significantly outperforming the control group (WG = 24.79 ± 0.26, SGR = 1.07 ± 0.02, $P < 0.05$). Key genera linked to each diet were Burkholderiaceae and Weizmannia (YS_MOS), Enterobacterales and Pseudoalteromonas (SSF2), and Escherichia and Cytophagales (control). PICRUSt functional analysis revealed predominant pathways linked to amino acid and fatty acid biosynthesis as well as organic compound degradation. The Shannon index positively correlated with the Apparent Digestibility Coefficient (ADC) for phosphorus, protein, and lipids, as well as nutrient retention and growth rate. These results indicate MOS enhanced gut bacteria growth and nutrient digestibility; SSF enhanced feed efficiency from the breakdown of antinutrients; and YS promoted gut health and nutrient absorption. Combined feed additives, particularly SSF_MOS, showed great potential for promoting growth and feed efficiency in cultured fish.



# CONTROL OF PARASITIC SALMON LICE (*Lepeophtheirus salmonis*) THROUGH OPTICAL LASER-DELOUSING IN NORWEGIAN SALMONID AQUACULTURE

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## Introduction

Parasitic salmon lice (*Lepeophtheirus salmonis*) present a significant challenge for salmonid aquaculture in Norway. Allowable lice abundance in commercial farms is strictly regulated, and farmers must engage in extensive delousing operations to adhere to regulatory thresholds. The widespread development of resistance to most available treatment chemicals has driven the adoption of mechanical treatment alternatives, many of which negatively impact growth, health, and welfare of farmed fish. Controlling measures that effectively reduce salmon lice without negatively affecting health and quality of farmed fish may therefore increase profitability and reduce the environmental footprint of current farming practices. Optical delousing is an alternative method of lice control that does not require handling of the fish. The system utilizes machine vision and object detection algorithms to identify and remove mobile and adult sea lice stages with targeted laser pulses on freely swimming fish. It relies on mobile hardware components that are continuously deployed in commercial sea cages, where they are actively positioned close to the fish. Here, we empirically evaluate the need for lice treatments at sites with and without optical delousing.

## Materials and Methods

Operational data on lice treatments and lice abundance are publicly available for all aquaculture sites in Norway. We grouped public data from all reportable sites along the Norwegian coast in 2023 into production weeks and added whether optical delousing was used during each production week. To capture possible effects of optical delousing on treatment frequency, we selected generalized linear mixed effects models (GLMEs) and estimated the weekly probability of a treatment taking place, considering sea temperature and the presence of optical delousing as predictors.

## Results

Results showed that sites relying on optical delousing represented 10.9% of production weeks, but only 5.9% of treatment weeks. Adjusted for a sea temperature of 10°C, sites without optical delousing had a 10.1% weekly probability to require treatment. For sites with optical delousing, the weekly probability to require other forms of sea louse treatment was significantly reduced by 50% to 5.06%. These differences persisted despite a lower reliance on cleaner fish at sites with optical delousing and in the absence of any indication of an accompanying increase in lice abundance at these sites.

## Conclusion

In conclusion, we show that optical delousing can be an effective tool to control lice abundance in salmonid aquaculture along the Norwegian coast without stressful handling of the fish. This may both benefit the health of farmed fish and reduce lice infection pressure on wild salmonid populations.

# REARING STRESS IMPACTS THE ENDOCRINE REGULATION OF GROWTH AND MOLTING IN LITOPENAEUS VANNAMEI (BOONE, 1931)

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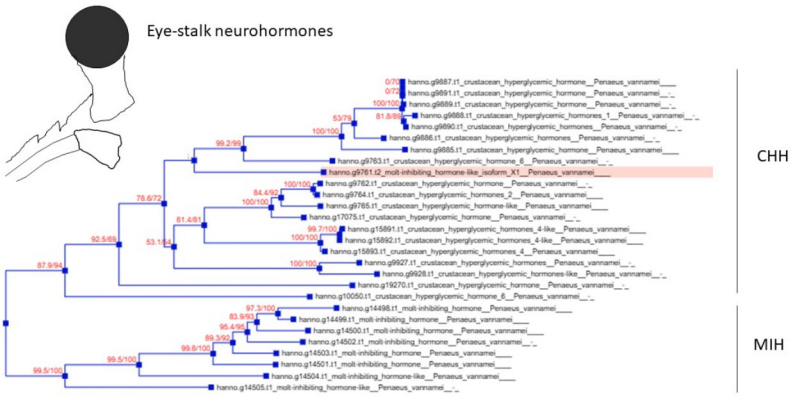
Introduction:
Shrimp farming rapidly increased over the past decade, currently comprising 6.8 billion t in Whiteleg shrimps Litopenaeus vannamei alone [1]. At the same time, concerns about the animal welfare have been raised and legislation on farming has been constantly adapted in several countries. Indeed, suboptimal rearing conditions are often not noticed due to lacking criteria and methods [2, 3]. In the interdisciplinary project CrustaWohl we studied stress-related behaviors, easy-to-monitor morphological abnormalities and injuries as well as the molecular basis of chronic stress, focusing on the eye-stalk as endocrine center regulating growth and molting in crustaceans.

Material and Methods:
After three weeks of acclimatization, juvenile Whiteleg tiger shrimps (14.73 ± 0.36 g) were transferred to twelve 600L tanks arranged in a RAS (water exchange one V/h; water renewal 2%/d) with a biofilter, UV disinfection, protein skimmer, drum filter and an ozone generator, providing three experimental groups reared at different stocking densities (low density (LD): 1 kg/m$^2$, medium density (MD): 2kg/m$^2$, high density (HD): 4 kg/m$^2$) and assessed in 4 replicates each.

For sampling, shrimps were euthanized in an ice slurry solution, weight and length were recorded and the molting stage was determined according to Robertson et al. (1987) with a binocular. Only shrimps in the intermolt and early premolt (D0-D1-D2) stages were selected. The eye-stalk was dissected and total RNA was extracted as described in Gamberoni et al. (2025). Library construction and RNAseq was performed by Novogene (Germany). RNAseq reads were mapped by hisat2 and expression analysis was done by cuffdiff using 5 biological replicates for each condition.




Neurohormones identified by RNAseq analysis. The CHH/MIH upregulated in response to chronic stress is marked in red.

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Results and Discussion:

In the eye-stalk as endocrine center for the regulation of growth and molting we identified 28 neurohormones of the crustacean neurohormone family, including 20 crustacean hyperglycemic hormones (CHH), 8 molt inhibiting hormones (MIH). Among all differentially expressed genes in the eye-stalk, a CHH formerly identified as MIH was up-regulated in the HD group. In crustaceans, the CHH controls blood sugar but also plays a role in molting. Interestingly, the resulting inhibition of growth may thereby shift energy to compensate for the persistent stressor and may function similarly to cortisol in vertebrates increasing plasma sugar in response to stress. Also, a significant growth inhibition was observed at progressively increasing density - both in the HD and MD groups - congruent with this hypothesis. As a conclusion, we believe that this CHH/MIH inhibits growth and molt and regulates hemolymph sugar, shifting energy to compensate chronic stress impact.

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# IMPLEMENTING THE STRATEGIC GUIDELINES FOR EU AQUACULTURE: DOCUMENT ON "GOOD HUSBANDRY PRACTICES FOR AQUACULTURE" AND "CODE OF GOOD PRACTICES ON FISH WELFARE AND FISH WELFARE INDICATORS"

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**Introduction**

This abstract introduces two documents developed to support the European Commission's (EC) "*Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030*"[1]. The two documents are the result of a desk study and an extensive consultation process with scientific experts and stakeholders including the Aquaculture Advisory Council (AAC).

**Methodology**

The "Good Husbandry Practices for aquaculture" document was developed by the EU Aquaculture Assistance Mechanism (AAM) through extensive data collection. It is a desk study and a literature review on published reports and peer-reviewed papers on EU and international regulations, standards and industry guidelines. In addition, an online survey targeting EU member states and the AAC, yielding 21 responses was conducted. Expert input was gathered through a cluster meeting with relevant EU-funded project representatives and AAM Board of Ambassadors members, leading to a second survey with 4 additional contributions and the identification of criteria for prioritizing information sources. Bilateral meetings were held with the Aquaculture Stewardship Council (ASC), the European Forum of Farm Animal Breeder (EFFAB), experts from the "Comité National de la Conchyliculture" (CNC), and a workshop with member state experts was organized by the European Commission. Furthermore, technical seminars with member state aquaculture experts and feedback from various associations and experts contributed to the document. The final good husbandry practices (GHPs) selection prioritized concrete industry examples, standards, and relevant project results.

The document on "Code of Good Practices on fish welfare and fish welfare indicators" was developed based on the "Document on Good Husbandry Practices" published on the EU aquaculture website in 2024 and the EU AAM's work on fish welfare good practices and indicators based on relevant data collections. This involved desk research and literature reviews published by international organizations on standards, and industry guidelines from EU member states, Norway, and the UK. Furthermore, an online survey targeting these countries and relevant stakeholders yielded 36 responses. The preliminary findings were then discussed and refined through an expert consultation meeting with representatives of relevant EU-funded projects and AAM Board of Ambassadors members, and the final document was reviewed by the Directorate-General for Health and Food Safety (DG SANTE) and experts from the European Union Reference Centre for Welfare of Aquatic Animals (EURCAW-Aqua).

**Document "Good husbandry practices for aquaculture"**

This document systematically outlines a range of critical husbandry practices essential for maintaining and enhancing the health and welfare of farmed aquatic animals throughout their production cycles. This extends beyond the explicitly mentioned "environmental enrichment (EE)," "functional feed (FF)," and "selective breeding (SB)" in the EU's "Strategic Guidelines for Aquaculture 2021-2030", encompassing fundamental aspects such as feeding regimes (optimizing quantity, rate, and distribution), handling procedures (minimizing stress and physical trauma), mortality management (efficient and hygienic removal), fallowing strategies (disease and parasite control), predator mitigation (prioritizing non-lethal methods), biosecurity protocols (minimizing escapes and disease transmission), water quality management (maintaining species-specific parameters), stocking density optimization (balancing welfare and production), disinfection and cleaning protocols (disease prevention), and prophylactic and therapeutic interventions (vaccination and treatments under veterinary supervision). These multifaceted topics are evaluated through the prism of the widely recognized five welfare domains: nutritional adequacy, environmental quality,

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health status, behavioral expression, and overall mental state, with the overarching aim of ensuring a high standard of life for farmed aquatic organisms. It systematically maps and compiles a non-exhaustive repertoire of general and species-specific good husbandry practices (GHPs) pertinent to the welfare and health of key European aquaculture species across diverse production methodologies. This compilation, encompassing species such as Common Carp (*Cyprinus carpio*), Rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*), Gilthead sea bream (*Sparus aurata*), Mussels (*Mytilus* spp)., and cleaner fish (*Labrus bergylta* and *Cyclopterus lumpus*) aims to provide practical support to operators in meeting and potentially exceeding fundamental legal obligations concerning animal health, welfare, and environmental impact, with a commitment to future updates incorporating additional good practices and relevant indicators. Chapter 4 presents 40 general GHPs and Annex II provides species-specific GHPs for the following species: Common Carp: 19, Rainbow trout: 22, Atlantic salmon: 27, European sea bass: 20, Gilthead Sea bream: 24, Mussels: 8, Ballan wrasse: 6, Lumpfish: 10.

**Code of good practices on fish welfare and fish welfare indicators**

This document serves as a complementary resource to the first GHP document. It delineates non-species-specific best practices for the welfare of farmed fish and the indicators employed in its assessment throughout various ontogenetic stages and production techniques. Developed through a collaborative effort between the EU AAM and the EURCAW-Aqua, this Code functions as a practical and accessible guide for aquaculture producers. Its focus is deliberately restricted to explicit welfare considerations, excluding aspects of fish health. The document details specific good practices related to these topics, feeding, handling, grading, water quality (including physicochemical and microbiological parameters), stocking density, mortality removal, and transport (pre-transport, loading/unloading, and during transport), totaling seventy-three distinct practices aimed at improving fish welfare in aquaculture. The document provides detailed expositions of each GHP topic, elucidating its significance for animal well-being. Chapter 4 outlines general, non-species-specific good practices for fish welfare in aquaculture, addressing topics from the preceding chapter. This Code introduces in Chapter 5 thirteen general, non-species-specific indicators relevant to the key husbandry topics. These indicators are crucial for monitoring animal well-being during critical production phases, including rearing, transport, and slaughter, enabling evidence-based evaluation of current practices and the implementation of targeted improvements. The document reviews existing scientific efforts and tools for welfare assessment, emphasizing the ongoing need for standardized, technology-driven, and universally applicable monitoring protocols to enhance the reliability and comparability of welfare evaluations across the EU aquaculture sector.

**Conclusion**

Both documents are fundamentally aligned with the objectives of the EU's "Strategic Guidelines for Aquaculture 2021-2030", sharing a common goal of augmenting the sustainability and competitiveness of the European aquaculture sector through evidence-based guidance derived from broad stakeholder engagement. The "Good Husbandry Practices" paper provides species-specific recommendations for health and welfare management, whereas the "Code of Good Practices" offers overarching, non-species-specific principles and indicators specifically for fish welfare, representing a concerted effort towards elevating animal welfare standards and fostering responsible practices within the European aquaculture industry.

# FUNCTIONAL DIVERSITY OF METHYL-END DESATURASES EN ANNELIDA REVEALS THEIR POTENTIAL FOR SUSTAINABLE LC-PUFA PRODUCTION IN AQUACULTURE

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**Introduction**

Long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) are essential nutrients with critical roles in neural function, immune regulation, and cellular membrane structure (Tocher et al., 2019). With increasing demand for sustainable LC-PUFA sources in aquaculture, especially for formulating nutritionally enriched feeds, exploring new biological systems capable of de novo LC-PUFA synthesis has become a critical research priority (Tocher et al., 2019). While vertebrates predominantly rely on dietary intake for LC-PUFA, certain invertebrates, including aquaculture-relevant annelids such as *Hediste diversicolor* and *Platynereis dumerilii*, possess endogenous biosynthetic pathways involving methyl-end desaturases (ωx), front-end desaturases (Fed), and elongases (Elovl) (Monroig et al., 2022). Among these, the diversity and functionality of ωx desaturases in Annelida remain underexplored (Kabeya et al., 2018). This study investigated the diversity and function of ωx desaturases in annelids across three major classes in Annelida: Polychaeta, Clitellata, and Sipuncula.

**Materials and Methods**

The sequences of ωx desaturase genes were retrieved from available annelids' genomic and transcriptomic databases. Conserved motifs were identified, and phylogenetic relationships were established. The functions of representative genes were heterologously expressed in yeast. The ωx desaturation activity was evaluated through substrate feeding assays and gas chromatography-mass spectrometry (GC-MS) fatty acid profiling.

**Results and Discussion**

A total of 109 sequences of ωx desaturase genes were isolated from annelids. Phylogenetic analyses grouped them into two main desaturase groups (A and B) (Figure 1). Functional characterisation revealed distinct enzymatic activities of five ωx desaturase genes from two representative species, *Eisenia fetida* and *Sipunculus nudus*: *E. fetida* ωx1 and *S. nudus* ωx1 exhibited Δ12 desaturase activity, catalysing the conversion of 18:1ω9 to 18:2ω6, whereas *E. fetida* ωx2, *S. nudus* ωx2, and *S. nudus* ωx3 demonstrated ω3 activities (Δ15, Δ17, and Δ19 activities), transforming ω6 substrates into their corresponding ω3 fatty acids, including eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). The number of ωx desaturase genes varied among species in Annelida. While most species typically possessed two desaturases—one Δ12 and one ω3—some species harbored 0, 1, 3, or even 4 ωx desaturase genes. The study confirms that annelids harbor diverse ωx desaturases capable of producing a broad range of LC-PUFA. The taxonomic distribution and functional plasticity of these enzymes suggest that annelids represent a valuable but underexploited resource for sustainable omega-3 fatty acid production. This work provides a foundation for future biotechnological exploitation of annelids in aquafeed formulation and contributes to circular economy models in aquaculture.

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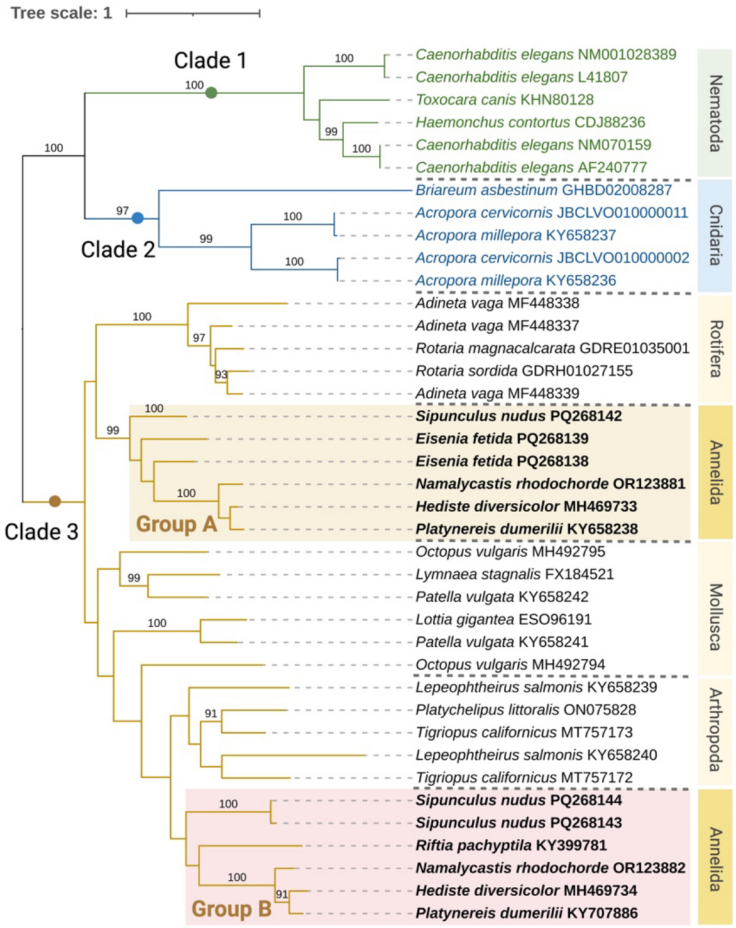


**Figure 1.** The phylogenetic tree of wx desaturase enzymes in Metazoa shows the evolutionary relationship between Annelida and other phyla of animals (Nematoda, Mollusca, Rotifera, Anthropoda, and Cnidaria). Clades 1, 2, and 3 were classified according to Kabeya et al. (2018). The numbers above indicate branches in the phylogenetic tree with support values equal to or exceeding 90%.

**Acknowledgements**

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# DIETARY INCLUSION OF FISH OIL ETHYL ESTERS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND LIPID METABOLISM IN EUROPEAN SEABASS (*Dicentrarchus Labrax)*

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**Introduction**

Due to declining fish oil (FO) availability, sustainability concerns, and rising costs, the increasing demand for sustainable aquaculture requires replacing FO with alternative lipid sources such as vegetable oils (VO). To support this shift, the aquafeed sector increasingly aligns with the "Farm to Fork" Strategy, part of the broader European Union Green Deal. However, marine fish have a limited capacity to elongate and desaturate C-18 fatty acids and, therefore, require a dietary source of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) to maintain growth and health. Ethyl esters (EE) oil, a by-product of human omega-3 supplement production from FO, is a promising source of n-3 LC-PUFAs. In the process of FO refining for n-3 LC-PUFA extraction for supplements to be used by humans, about 70% of FO is discarded as an EE oil by-product. This oil is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (around 5-7%), cholesterol, and vitamin D, offering advantages over VO as a lipid source for marine fish. However, EE oil may not be as bioavailable as FO and VO triglycerides. Therefore, this study aimed to evaluate the effects of dietary inclusion of EE oil as a partial or total replacement of FO in European seabass diets.

**Materials and Methods**

Four isoproteic (48% crude protein) and isolipidic (16% crude lipid) practical diets were formulated, differing in lipid sources, as follows (% total lipid inclusion):

Diet 1 (D1): 72% FO, 28% rapeseed oil (RO), and 0% EE oil;
Diet 2 (D2): 28% FO, 72% RO, and 0% EE;
Diet 3 (D3): 28% FO, 44% RO, and 28% EE;
Diet 4 (D4): 0% FO, 28% RO, and 72% EE.

Chromic oxide was added as an external digestibility marker to the diets.

The experiment was performed at CIIMAR, Matosinhos, Portugal, and consisted of a growth trial and a digestibility trial. The growth trial was performed in a recirculating aquaculture system (RAS) at 23ºC for 88 days. Each diet was randomly assigned to three replicates, with fifteen European seabass (*Dicentrarchis labrax*) juveniles (initial body weight: 62g) per tank. Five fish from the initial stock population and three fish per tank at the end of the growth trial were euthanized (overdose of 2-phenoxyethanol), and the weight of the fish, liver, and viscera were recorded. Fish were then stored at −20 °C for whole-body composition analysis. The digestibility trial was also conducted at 23ºC in the RAS. Twelve groups of ten fish with an average weight of 161 g were established, and feces were collected over 15 days. Statistical analysis of the data was performed using one-way ANOVA. When $p < 0.05$, means were compared with Tukey's multiple-range test. All analyses were done with SPSS 29.0 software for Windows (IBM® SPSS® Statistics, New York, USA).

**Results**

Fish readily accepted the diets, and mortality during the trial was low and unaffected by diet composition. Final body weight, weight gain, and daily growth index showed no significant differences between dietary treatments. Feed intake, feed efficiency, protein efficiency ratio, nitrogen, and energy retentions (% intake) were also unaffected by dietary treatments. The hepatosomatic index and visceral somatic index, and the whole-body composition (dry matter, protein, lipid, ash, and energy) were also unaffected by dietary treatments. Moreover, the apparent digestibility coefficients (ADC) for dry matter, protein, lipid, and energy were similar between diets.

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## Conclusion

The present results indicate that EE oil can be included in diets for European seabass without compromising growth performance, feed utilization, or nutrient digestibility, highlighting its potential as an alternative to FO in aquafeeds.

## Acknowledgments

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# EFFECTS OF DIETARY INCLUSION OF FISH OIL ETHYL ESTERS ON FATTY ACID PROFILE, PLASMA METABOLITES, AND LIVER OXIDATIVE STRESS IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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## Introduction

The nutritional quality of dietary lipids plays a critical role in regulating physiological functions by modulating tissue fatty acid composition, metabolism, inflammatory and immunological responses, and cellular oxidative balance. The selection of dietary lipid sources influences growth performance and key health-related responses. In marine fish, fish oil (FO) remains the standard lipid source for providing essential n-3 LC-PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, alternative lipid sources have emerged as potential substitutes. Ethyl esters (EE) oil is a sub-product of omega-3 production for human consumption and may be valorized as an alternative lipid source in the context of a circular economy. EE oil differs from FO in molecular structure, which may alter its biological efficacy. Marine fish species rely on dietary sources of n-3 LC-PUFA to meet fatty acid requirements, as they cannot synthesize it from C18 precursors. Thus, understanding how EE oil impacts lipid metabolism and redox status is essential for optimizing feed formulations. This study explores the effects of partial or total FO replacement with EE oil on the fatty acid profile, plasma metabolites, and hepatic oxidative stress of European seabass (*Dicentrarchus labrax*), an important species for Mediterranean aquaculture.

## Materials and Methods

Four isonitrogenous (48% crude protein) and isolipidic (16% crude lipids) diets were formulated by varying the proportions of FO, rapeseed oil (RO), and EE oil in the total lipid blend, as follows (% total lipid inclusion): D1 (72% FO, 28% RO, 0% EE; EPA+DHA=12.8% FA), D2 (28% FO, 72% RO, 0% EE; EPA+DHA=4.2% FA), D3 (28% FO, 44% RO, 28% EE; EPA+DHA=4.9% FA), and D4 (0% FO, 28% RO, 72% EE; EPA+DHA=3.6% FA). Juvenile European seabass (*Dicentrarchus* labrax) with ~ 62 g initial body weight were randomly distributed into three replicate tanks under controlled temperature (23°C) in a RAS system for 88 days. Fish were fed to apparent satiation twice daily. At the end of the trial, blood, muscle, and liver samples were collected from three fish per tank for biochemical, fatty acid profile, and oxidative stress analysis. Statistical differences among treatments were evaluated by one-way ANOVA and Tukey's test when $p < 0.05$, using SPSS 29.0 (IBM® SPSS® Statistics, New York, USA).

## Results

The zootechnical performance of the animals was not the aim of this study, and the results are presented in another work at this conference. Briefly, diet composition did not affect growth performance and whole body composition. The fatty acid profile of muscle and liver tissues reflected dietary lipid sources and exhibited statistically significant differences. In the muscle, α-linolenic acid (ALA, C18:3n-3) levels were significantly higher in fish fed the D2 and D3 diets compared to D4 and D1 diets. However, EPA (C20:5n-3) levels were significantly higher in fish fed the D4 diet than in D2 diet, whose values were comparable to D1 diet. DHA (C22:6n-3) was highest in fish fed the D1. In the liver, saturated fatty acids (SFA), such as C14:0 and C16:0, were significantly increased in fish fed the D4, leading to the highest total SFA, while D2 showed the lowest level. Monounsaturated fatty acids (MUFA), particularly C18:1n-9, were significantly increased in fish fed the D2. Plasma glucose concentration was significantly higher in fish fed the D2 than in D3 diets. In contrast, total protein, triglycerides, total lipids, and cholesterol remained unaffected by dietary treatments. Liver oxidative stress biomarkers showed that glutathione reductase activity and lipid peroxidation levels were significantly lower in fish fed the D4 than in the D1 diets. No significant differences were observed for glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD) among the dietary treatments.

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## Conclusion

EE oil inclusion in European seabass juvenile diets altered tissue fatty acid profiles, reducing DHA and total n-3 PUFA compared to a FO-based diet. However, EPA levels were maintained, and hepatic lipid peroxidation was reduced. Overall, these results suggest the potential for dietary inclusion of EE oil in marine fish diets.

## Acknowledgments

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# EFFECTS OF FISH OIL ETHYL ESTERS ON BODY COMPOSITION AND LIVER OXIDATIVE STRESS IN EUROPEAN SEABASS (*Dicentrarchus labrax*) UNDER THERMAL CHALLENGE

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**Introduction**
Marine heatwaves driven by climate change threaten aquaculture sustainability in Mediterranean species such as European seabass (*Dicentrarchus labrax*) by disrupting physiological homeostasis, particularly metabolism and oxidative balance. Nutritional strategies to improve thermal resilience are increasingly needed. Fish oil (FO), rich in n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), faces sustainability challenges and is increasingly being directly used for human consumption. Ethyl esters (EE), by-products from FO refining for omega-3 supplement production, have recently emerged as a potential alternative lipid source. EE oil is rich in EPA and DHA, which are present as ethyl esters rather than triglycerides, and this may influence bioavailability under heat-stress conditions. Thermal stress can alter membrane fluidity, whose composition is modulated by the dietary lipid source. Previous studies have shown that EE oil has potential as a lipid source under normal temperature conditions, but its impact during thermal challenges has not yet been explored. This study evaluated the effects of dietary EE oil on body composition, fatty acid profile, and liver oxidative stress in seabass under simulated heatwave conditions.

**Materials and Methods**
Four diets were formulated as isoproteic (48% crude protein) and isolipidic (16% crude lipid), and the lipid sources (% total lipid) were as follows: D1 (72% FO, 28% rapeseed oil, RO; EPA+DHA=12.8% FA), D2 (28% FO, 72% RO; EPA+DHA=4.2% FA), D3 (28% FO, 44% RO, 28% EE; EPA+DHA=4.9% FA), and D4 (28% RO, 72% EE; EPA+DHA=3.6% FA). Firstly, a growth trial was performed at 23 ºC for 88 days with European seabass (*Dicentrarchus* labrax) juveniles with an initial weight of 62g. Then, a thermal challenge was imposed in two phases: 29 ± 0.5 °C for 7 days, followed by 31 ± 0.5 °C for 7 days. At the trial end, three fish per tank were euthanized (overdose of 2-phenoxyethanol) for measurement of body, liver, and viscera weights for hepatosomatic index (HSI) and viscerosomatic index (VSI), and whole-body composition (dry matter, crude protein, lipids, ashes, and energy). Liver and muscle were sampled from 3 other fish per tank to assess fatty acid profiles, and liver oxidative stress biomarkers, including glutathione peroxidase (GPX), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), and lipid peroxidation (LPO). Data was evaluated by one-way ANOVA and Tukey's test ($p < 0.05$) using SPSS 29.0 software for Windows (IBM® SPSS® Statistics, New York, USA).

**Results**
Under the thermal challenge, dietary treatments had no significant differences in whole-body dry matter, protein, lipids, ash, gross energy content and VSI. The HSI was significantly higher in D4 than in D2. Fatty acid profiles of muscle and liver reflected dietary lipid composition: DHA and total n-3 PUFA levels were significantly reduced in both tissues of fish fed the D4 diet. In muscle, EPA was higher in D4 than in the D2 and D3 diets but remained lower than in the D1. In the liver, EPA, DHA, and total n-3 PUFA were significantly reduced in fish fed the D4 diet compared to the D1 diet. In contrast, saturated fatty acids (SFA) increased significantly in D4, resulting in the highest SFA levels among all dietary treatments. Liver oxidative stress enzymes and LPO levels did not differ significantly among dietary treatments.

**Conclusion**
In conclusion, dietary EE oil inclusion altered tissue fatty acid composition, particularly reducing n-3 LC-PUFA and increasing SFA levels, without markedly affecting whole-body composition and oxidative stress responses under thermal challenge.

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**Acknowledgments**
This work was supported by the Zero-Waste Omega-3: A Green Label Solution for Sustainable Aquaculture Feeds project, funded by the Portuguese Foundation for Science and Technology (Ref: 2023.13017.PEX; DOI:https://doi.org/10.54499/2023.13017.PEX). Yue Xu was supported by a CSC scholarship, funded by national resources from the Chinese government (Ref: 202108310020). Rui Magalhães received funding through a CEEC Individual contract (Ref:2023.09082.CEECIND/CP2848/CT0017; DOI:https://doi.org/10.54499/2023.09082.CEECIND/CP2848/CT0017). EE oil was supplied by Epax® Norway AS, Ålesund, Norway.

# BREED ABOVE THE CURVE: QUANTITATIVE GENETICS FOR SHAPE-RELATED TRAITS USING IMAGE ANALYSIS IN GILTHEAD SEABREAM (*Sparus aurata*)

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## Introduction

Body shape is a critical trait in aquaculture breeding and management, as it influences both the biological performance and the market value of fish. Body shape can vary considerably due to environmental conditions, and can be influenced by artificial selection. Shape can therefore serve as an indicator to study environmental adaptation of certain species. For species that are marketed as whole, shape is particularly important as it influences consumers' preference and the perceived quality of the product (Colihueque & Araneda, 2014).

However, the definition and quantification of body shape remains complex. Shape is not a single trait but a collective expression of various morphological attributes. In flatfish such as sole, shape can often be approximated with simple geometric measurements such as fitting an ellipse. For finfish such as gilthead seabream, shape quantification becomes more challenging and typically relies on multiple indicators derived from landmarks, ratios or manually annotated traits (Fragkoulis et al., 2021). These indicators might exhibit low genetic correlation with the shape outline and are not favoured by breeders for indirect selection.

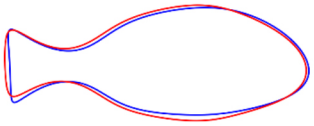

**Figure 1** Blue: average best contour; Red: average worst contour.

Besides quantitative measurements, shape is also assessed in breeding programs through expert grading, where visual scores are assigned based on pre-defined criteria. While this method reflects practical and market driven evaluations, it might suffer from subjectivity and low resolution (Blonk et al., 2010), restricting the coefficient of variation available for selection.

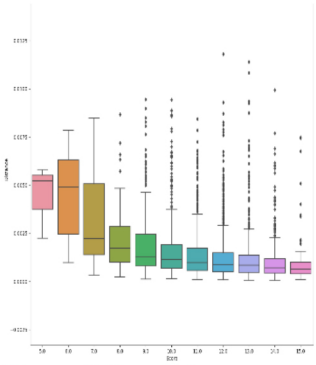

**Figure 2** Correlation between scores and DtB. X-axis: the combined score of all traits, ranging from 5 to 15; Y-axis: DtB value.

To overcome these limitations, we propose an analytical pipeline for automated quantitative analysis of the body shape in gilthead seabream (Sparus aurata). Based on the manual scores on selection candidates and their images, we defined a "Best" outline and a "Worst" outline and subsequently derived two novel traits for selection candidates: Distance to the Best (DtB) and Distance to the Worst (DtW). These traits quantify how closely each individual resembles the most, or least, desirable shape. Furthermore, we demonstrate that both traits exhibit genetic variance and can be integrated into breeding programs for shape improvement.

## Materials

Images were collected in 10 batches at harvest from 3,421 gilthead seabream (*Sparus aurata*), all of which were breeding candidates with known pedigree information. Each fish was visually scored on five morphological traits: head, ventral side, peduncle fin, caudal peduncle, and caudal side. Scores ranged from 1 to 3, with a score of 3 indicating the ideal condition for each trait.

## Methods

For each image the fish was segmented from the background using the Segment Anything Model (SAM). Contour detection was then performed using OpenCV, and Elliptic Fourier Descriptors (EFD) were applied to mathematically transform the extracted contours. Each contour was reconstructed using seven harmonics of the EFD, producing a smooth outline with 1,000 key points.

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The reconstructed contours were standardized in orientation using Principal Component Analysis (PCA), aligning them according to their first and second principal components. To ensure consistency, all key points on the contours were re-indexed starting from the anterior tip and proceeding clockwise.

An average contour was generated via Procrustes analysis using only the fish that received a score of 3 for all traits. The Procrustes distance from each individual's contour to this average was calculated and defined as a new trait: **Distance to the Best (DtB)**. The same process was repeated using contours from fish that received a score of 1 for all traits, producing a second trait: **Distance to the Worst (DtW)**. All image processing and distance calculations were conducted in Python 3.9.

Heritability estimates for DtB and DtW were obtained using a univariate animal model, and their genetic correlations with the original visual scores were calculated using a bivariate model. All genetic analyses were performed using ASReml-R.

**Results**

Figure 1 illustrates the average contours for the best-scoring and worst-scoring fish. The heritability estimates were $0.39 \pm 0.04$ for DtB and $0.27 \pm 0.03$ for DtW.

We evaluated the agreement between DtB and the original visual scores, with a particular focus on DtB. As shown in Figure 2, there was a general negative correlation between DtB and the manual scores, with a significant Pearson correlation of -0.12, indicating that higher-scoring fish tended to be closer to the ideal contour, with a smaller DtB value.

Genetic correlations of DtB and DtW with the original trait scores ranged from low to moderate. For example, the dorsal side score showed near-zero genetic correlation with both DtB and DtW, suggesting limited variance or low consistency in the manual grading for this region. In contrast, the ventral side score exhibited moderate genetic correlations: $-0.36 \pm 0.08$ with DtB and $0.27 \pm 0.03$ with DtW, indicating greater phenotypic and genetic variance in that area. This trend is visually supported by the contour plots in Figure 1, where the greatest divergence between best and worst shapes occurs along the ventral region. In comparison, the dorsal contours appear more aligned and parallel, suggesting lower shape variance and scoring sensitivity in that region.

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# A VIRTUAL FARM TO SUPPORT CLIMATE CHANGE ADAPTATION STRATEGIES IN SALMON AQUACULTURE

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**Introduction**
Climate change impacts farmed salmon in open net-pens through stressors like increased temperature, pH and storms. To adapt, the industry needs robust tools that can help to better understand complex interactions between the farm environment and production factors. This study developed a dynamic simulation tool (Virtual Farm) which can serve as a digital representation of salmon production in open net-pen system, allowing for experimentation and analysis of management and climate adaptation strategies. The criteria for comparing between different strategies are set by users based on the chosen performance indicators such as dissolved oxygen (DO) level in the net-pen, standard feeding rate, disease risk period and average daily weight gain by the fish.

**Methods**
The Virtual Farm model was constructed using Vensim DSS 10.2.1 simulation software. The main components and feedback structure of the model were first captured in a causal loop diagram (CLD), based on information gathered from both stakeholder engagements and the literature. Then following the CLD, the Virtual Farm model was developed using stocks, flows, feedback loops and time delays that are connected using mathematical relationships derived from knowledge and data about real farm system interactions. The differential equations in the model are solved using Euler integration method, with a one-day simulation time step to reflect daily and seasonal dynamics of the model parameters. Data loggers were deployed at three salmon farm locations on the west coast of Scotland and used to collect data on sea temperature and dissolved oxygen (DO) for model calibration and validation.

The model requires the user to input time-series data for seawater temperature, salinity, wind speed, chlorophyll-a, and water current speed as well as initial values for DO and production related factors like stocking weight, to simulate DO dynamics in the farm environment. The farm environment determines how an individual fish in a net-pen would respond in terms of feed intake, metabolism, growth, and then scaled up to the population level to simulate overall implications for farm production (total biomass and potential risk periods).

**Results and Discussion**
Results show that the modelled DO compare well to measured data at the three farm locations. Further model testing and refinement is underway through an iterative process and consultation with industry stakeholders.

The Virtual Farm model is designed to simulate the effects of multiple stressors (environmental and operational) and can help farmers and researchers to better understand and respond to climate change impacts on salmon production.

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# THERMAL STRESS AND CLIMATE-DRIVEN DISTRIBUTIONAL SHIFTS IN *Turbo sazae*: INTEGRATING IMMUNOLOGICAL AND GENETIC PERSPECTIVES

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*Turbo sazae*, a commercially and ecologically important marine gastropod historically found around Jeju Island, has recently shown signs of northward range expansion along Korea's eastern coast, likely driven by rising seawater temperatures. Concurrently, populations in Jeju have experienced a marked decline, with potential links to environmental stressors remaining unclear. To better understand the biological responses of *T. sazae* to changing thermal environments, this study integrated cellular immunological analysis and genetic connectivity assessments. Experimental exposure to elevated seawater temperatures (22°C–30°C over 9 days) revealed significant physiological stress, including reduced hemocyte phagocytic activity and increased reactive oxygen species (ROS) production. In parallel, mitochondrial COI sequence analysis from six populations across Jeju and the East Sea indicated genetic connectivity potentially facilitated by ocean currents, with a dominant shared haplotype (EJ1) suggesting historical gene flow. Despite low genetic differentiation, ancient divergence between populations was inferred, emphasizing the importance of ongoing genetic monitoring. Together, these findings underscore the dual impact of thermal stress on immune function and potential climate-mediated range shifts in *T. sazae*, calling for comprehensive conservation strategies.

# COMPARATIVE STUDY ON THE EFFECTS OF DL-METHIONINE AND HMTBa AS DIETARY METHIONINE SOURCES: GROWTH PERFORMANCE, FEED EFFICIENCY, AND ANTIOXIDANT ACTIVITY IN SIBERIAN STURGEON

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This study evaluated the effects of DL-Methionine (DL-M) and 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) as dietary methionine sources on growth performance, feed efficiency, and antioxidant activity in Siberian sturgeon (*Acipenser baerii*).

Experimental diets were formulated using equimolar quantities of each methionine source, incorporated into a commercial basal diet. Fish were fed four times daily according to the manufacturer's guidelines for 75 days. A total of 560 sturgeon (initial weight 961.41 ± 73.27 g) were allocated to 14 outdoor pools (two treatments, seven replicates). Environmental conditions were maintained consistently throughout the study.

Results showed no significant differences in final body weight, weight gain, or feed conversion ratio between the DL-M and HMTBa groups ($p > 0.05$). However, serum catalase (CAT) activity and total antioxidant capacity (TAC) were significantly higher in the HMTBa group ($p < 0.05$), while glutathione peroxidase (GPx) activity was higher in the DL-M group ($p < 0.05$).

In conclusion, HMTBa was as effective as DL-Methionine in promoting growth performance and enhancing antioxidant activity in Siberian sturgeon. These findings suggest that HMTBa is a viable alternative methionine source for sturgeon aquaculture.

# REMOTE MONITORING OF OFFSHORE AQUACULTURE STRUCTURES USING LOAD CELL SENSORS

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## Introduction

Offshore aquaculture cages are increasingly subjected to harsh marine conditions such as strong currents, wave action, and storm-induced loads, which threaten their structural integrity and can lead to significant economic losses, fish escape, safety hazards, and environmental damage [1]. To address these challenges, this study presents a real system deployment of a structural health monitoring solution that uses load cell sensors to measure cage tension and communicates data remotely via satellite. By integrating real-time sensing with satellite communication, the system enables continuous monitoring of environmental dynamics and timely detection of stress events in offshore fish cages.

## Materials and Methods

The load cell sensor is deployed on offshore fish cage mooring lines to measure tension in real time. The sensor, securely anchored to the seabed, connects via a waterproof cable to a data logger housed on a floating buoy. Data collected by the sensor is transmitted through satellite communication to a cloud server, enabling remote access through a dedicated web interface. Figure 1 illustrates the physical components of the system, the data transmission flow, and includes photographs that depict the real deployment of the buoy, anchor, and sensors in the field.

## Results and Discussion

Figure 2 presents a time series of load measurements captured by the deployed monitoring system, specifically showing the tension exerted on the floating buoy anchoring point. These values, transmitted via satellite, reflect how the buoy responds to changing marine conditions such as waves, currents, and wind. Peak loads exceeding 250 kg were observed during periods of intense environmental activity, followed by a gradual decline and stabilization—likely indicating calmer sea states or repositioning of the buoy.

This real-time monitoring demonstrates the system's effectiveness in capturing dynamic forces acting on offshore infrastructure. Satellite-based communication ensures uninterrupted data delivery, enabling remote analysis and early detection of abnormal stress levels on the buoy and the fish cages. Such insights are critical for maintaining system stability, guiding preventive maintenance, and improving the resilience of floating structures in offshore aquaculture operations.

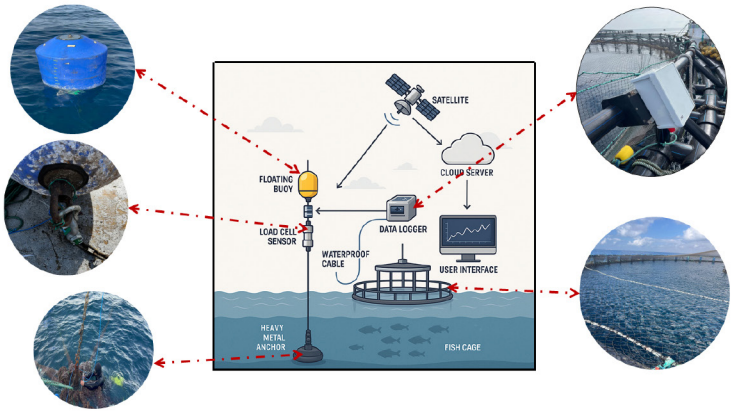


*Figure 1: Overview of the Smart Mooring Monitoring System and Its Components.*

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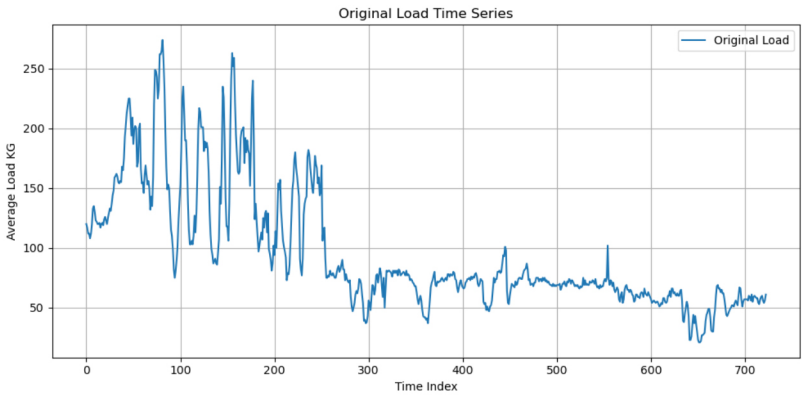


*Figure 1: An extract of remote Mooring Load Data Transmission and Monitoring Over Time.*

**Conclusion**

Our application demonstrates a reliable method for monitoring the structural health of offshore aquaculture in real time. The system facilitates prompt management actions to improve the resilience and sustainability of infrastructure by facilitating the early detection of stress events connected to tension. The sensor-driven monitoring is expected to be crucial for ensuring cage stability as aquaculture grows into more challenging marine settings and for facilitating long-term data collecting to create predictive models for proactive maintenance scheduling.

**Acknowledgement:** This study was funded by the AQUAMOORE project. Appreciation is extended to the project partners and the aquaculture companies that provided access to their facilities for this study.

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# RESOURCE RECOVERY AND INCREASING PRODUCTIVITY IN SUSTAINABLE INTENSIVE NEAR ZERO WASTE ASSIMILATION BASED RECIRCULATING AQUACULTURE SYSTEMS (AsRAS)

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Conventional aquaculture systems typically release 70–80% of the N, P and C from feed into the environment, primarily through dissolved and solid wastes, thereby contributing to significant pollution. As an alternative to traditional approaches relying on nitrification followed by water exchange or denitrification, assimilation-based recirculating aquaculture systems (AsRAS)—including aquaponics, algaponics, and bacterioponics—offer a more sustainable solution. All AsRAS couple conventional RAS with an assimilation-based reactor in a symbiotic arrangement, whereby fish-generated wastes serve as nutrients for plants (in aquaponics), algae (in algaponics), or bacteria (in bacterioponics) (Zhu et al., 2024; Yogev & Gross, 2019). This configuration enables the biological assimilation of fish waste, which purifies the water for recirculation while simultaneously maximizing water and nutrient use efficiency, minimizing effluent discharge, and recovering or conserving energy.

Over the past few years, we have developed three distinct AsRAS configurations that operate on fresh or brackish water in multi-loop, near-zero waste setups. Each system maintains separate loops for fish production and assimilation-organism growth, optimizing conditions for both products. Our experimental trials demonstrated fish stocking densities as high as 80 $kg/m^3$ (with a typical density of ~50 $kg/m^3$), survival rates exceeding 95%, and feed conversion ratios between 1.1 and 1.4. Notable energy savings were observed: over 80% in aquaponic systems and about 50% in biofloc-based RAS. Furthermore, carbon sequestration in aquaponics reached levels 1.4 times higher than the carbon content in feed, achieving a 64% reduction in carbon footprint relative to conventional RAS; greater reductions are anticipated in algaponics-based AsRAS. In all systems, $N_2O$ emissions were significantly lower than those from traditional RAS.

These findings are among the first to demonstrate the viability of large-scale AsRAS production with near-zero water and waste discharge, coupled with substantial energy savings. We posit that AsRAS hold considerable promise for replacing traditional nitrification–denitrification-based RAS, thereby advancing more sustainable aquaculture practices.

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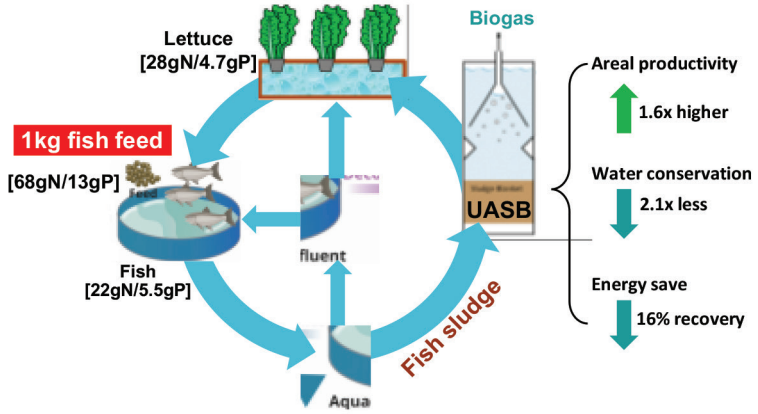
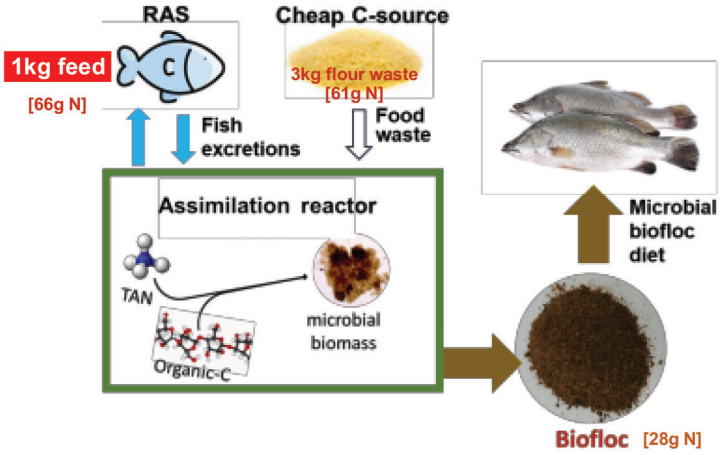
## Novel AsRAS: Coupling anaerobic digestion with aquaponics




## Novel AsRAS: A novel microaerophilic assimilation reactor with RAS



# COMMERCIAL-SCALE TREATMENT FACILITY (DENITRIFICATION) FOR LAND-BASED MARINE RAS EFFLUENTS

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To date, marine land-based recirculating aquaculture system (RAS) still include effluent stream discharge. These effluents, originating from fish metabolism, feed residues, and sludge washouts, are rich in nitrogenous and organic compounds. Due to their salinity, such streams can not discharge to freshwater bodies or municipal wastewater treatment plants and are typically released into the sea. However, environmental regulations on nutrient discharge into marine environments are tightening globally, particularly regarding nitrogen and suspended solids. This creates an urgent need for effective, scalable, and regulatory-compliant treatment solutions. Denitrification, particularly heterotrophic, is the prevailing approach for nitrate (Nitrogen) removal from saline effluents, yet commercial-scale applications remain scarce.

**System description**

A full-scale effluent treatment system was established and operated to treat the discharge from a semi-commercial marine RAS (Fig 1). Effluents consisted of solids filter backwashes, sludge removal by floor suction, and tank overflow. These were collected in a sump and pumped into a ~30 $m^3$ denitrification reactor based on the activated sludge concept. The reactor was coupled with a pressurized settler (Soliquator SLS700) with optional flocculant dosing for enhanced separation. Polished water was filtered through twin sand filters before sea discharge. Excess sludge was diverted to an anaerobic stabilization pond (450–750 $m^3$), followed by a ~30 $m^3$ aerobic mixed reactor in a secondary tank and optional recirculation to the anoxic stage.



Fig1. Picture of the semi-commercial marine- RAS effluent treatment (denitrification) facility

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# ADVANCING MULTI-LOOP AQUAPONICS: INTEGRATING ANAEROBIC DIGESTION FOR CIRCULAR, DESERT-ADAPTED FOOD SYSTEMS

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Aquaculture systems generate nutrient-rich effluents containing high loads of nitrogen, phosphorus, and organic matter, which can cause serious environmental harm if not properly treated. As a result, farmers are compelled to invest significant resources in waste management. Transforming these "wastes" into resources is a central challenge for sustainable aquaculture. Aquaponics—an integrated system that combines aquaculture with hydroponics—offers a promising solution by enabling the recovery and reuse of water, nutrients, and organic compounds. However, traditional one-loop aquaponics systems utilize only a fraction of the available nutrients, particularly those locked in solid waste (sludge). These systems are also limited by the tight coupling between the fish and plant units, which restricts the ability to optimize conditions independently and reduces system flexibility. Any malfunction in one component can negatively impact the entire system, compromising stability and performance.

This study presents a novel, zero-waste multi-loop aquaponics configuration designed to overcome the limitations of conventional one-loop systems. Our system integrates three independently optimized subsystems: (1) a recirculating aquaculture system (RAS), (2) deep-water hydroponics, and (3) anaerobic digestion units—one for fish sludge and another for plant residues. These digesters maximize nutrient mobilization and recovery. The system was deployed in a desert environment and operated under near-zero discharge conditions. Anaerobic supernatants were returned to the hydroponic loop as a nutrient-rich solution, while biogas was harvested as a renewable energy source.

Compared to traditional decoupled and coupled systems, the novel multi-loop system showed substantial improvements in resource recovery and overall productivity. Nutrient recovery rates for nitrogen and phosphorus reached 76% and 80%, respectively—up to twice the efficiency of standard one-loop designs. Plant productivity increased significantly, reaching 11.8 kg of lettuce per kg of fish feed, representing a 1.6-fold improvement over conventional systems. Furthermore, the system used 2.1× less water and required 16% less energy per kg of feed, with biogas production offsetting 84% of energy demand. Photosynthetic $CO_2$ sequestration further contributed to reducing the system's carbon footprint.

In summary, multi-loop aquaponics with integrated anaerobic digestion offers a scalable, circular, and resilient model for sustainable food production. By decoupling biological flows through engineered loops, this approach maximizes nutrient use, minimizes waste, enhances crop yields, and recovers may energy. These findings highlight its strong potential for sustainable agriculture in arid and urban settings where traditional farming methods are challenged.

# THE HIDDEN IMPACT OF INCREASING TEMPERATURES: HOW FISH LOSE THEIR RESILIENCE AGAINST OTHER STRESSORS

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## Introduction

Human activities and anthropogenic greenhouse gas emissions have driven climate change at unprecedented rates (IPCC 2014, 2019). Continued emissions will lead to further atmospheric and oceanic warming, triggering long-term shifts in the climate system that will exacerbate existing risks and introduce new challenges for all life on Earth.

Aquaculture is increasingly affected by climate-related stressors, including rising temperatures, more frequent extreme weather events, ocean acidification, and shifts in salinity and oxygen levels. The severity of these impacts will vary based on multiple factors, as will the sector's capacity for adaptation (Falconer et al., 2022). In Norwegian aquaculture, high mortality rates stem from various causes, including pathogens, parasites, ulcers, gill diseases, and the strain of intensive handling procedures. As climate change accelerates, and its effects become more pronounced, environmental conditions will emerge as a significant risk factor - intensifying known threats as well as introducing new challenges to fish health. Notably, in 2024, injuries caused by jellyfish rose to rank among the top three causes of mortality in farmed salmon in Norway. Additionally, francisellosis, caused by *Francisella noatunensis*, reappeared in farmed cod after years without reported cases.

Temperature projections indicate that many regions in Norway may experience elevated temperatures over the next 10–15 years, including more frequent days exceeding optimal thresholds for currently farmed species. Suboptimal environmental conditions can induce micro-damages at the molecular and cellular levels in important organs, compromising fish health (Ytteborg et al., 2022). While ongoing technological advancements and new production methods continue to enhance fish health, the impacts of climate change may mask the benefits of these innovations.

## Materials and methods

Using downscaled temperature projections based on the Intergovernmental Panel on Climate Change (IPCC) climate models (Shared Socioeconomic Pathways, SSPs), we analyzed potential future temperature conditions at existing and planned Atlantic cod farms in Norway. Regional climate projections were assessed using the Challenging Conditions Index (CCI), a method developed by Falconer et al. (2024) that evaluates environmental suitability based on a species' thermal preferences.

To simulate near-future scenarios, a temperature trial was conducted, exposing farmed Atlantic cod (*Gadus morhua*) to three temperature conditions: low (12°C), high (17°C), and fluctuating (12–17°C) temperatures. The robustness of cod reared at suboptimal temperatures was further tested by exposing fish to either jellyfish fragments or *F. noatunensis*. Skin and gill samples were collected from all temperature groups - including exposed and non-exposed individuals - and analyzed using histology and transcriptomics.

## Results and discussion

The evaluation of climate projections for future cod farms indicates that several locations may experience temperatures exceeding those considered suitable for cod. The Challenging Conditions Index (CCI) further suggests that farming conditions over the next 10–15 years could become increasingly difficult.

Findings from the experimental trial revealed that temperature alone induce micro-damages in the epidermis of both skin and gills. Exposure to high and fluctuating temperatures led to increased breaches between individual epithelial cells, alongside heightened expression of stress-related genes such as *sod*, *hsp70*, and *catalase*. Following exposure to jellyfish fragments or *F. noatunensis*, fish exhibited increased mucus production, elevated stress responses, and a higher frequency of morphological lesions - effects that were more severe under high and fluctuating temperature conditions.

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Both skin and gills are highly sensitive to unfavourable environmental conditions, making their morphological analysis valuable for assessing the impact of environmental stressors and identifying potential challenges. The observed damage may reduce fish resilience, impairing their ability to withstand climate change-induced stressors, secondary environmental pressures, and general production-related stress. These findings underscore the importance of evaluating future environmental conditions and species-specific tolerances prior to site selection for future cod farming.

**Funding**
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# PHYSIOLOGICAL AND METABOLIC RESPONSES OF COBIA (*Rachycentron canadum*) JUVENILES FED FISH OIL-FREE DIETS WITH DIFFERENT LC-PUFA/C18 PUFA RATIO

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**Introduction**

Fish oil (FO) has traditionally been used in marine organism diets as the main lipid source and key of essential long-chain polyunsaturated fatty acids (LC-PUFAs). However, its global availability has declined due to reliance on wild-caught marine fish and stagnant fishery production over the past three decades, making it unfeasible to support the fast growth of aquaculture with such a limited resource (Teles et al., 2015; Nordahl, 2011; Fao, 2024).

Alternative nutritional strategies aim to reduce dependence on limited ingredients like (FO) while ensuring safe and efficient diets for marine species. Replacing FO partially or entirely with vegetable oils is a common practice in aquaculture, but it changes the dietary n-3/n-6 ratio, potentially impairing immune responses, physiological functions, and growth performance in commercial fish species (Turchini et al., 2019; Magalhães et al., 2022).

Diet formulation should provide both adequate levels and an appropriate balance of fatty acids (FA), with particular emphasis on the dietary n-3/n-6 ratio, to optimize nutritional efficiency and the selection of lipid sources. Such strategies promote the development of healthier and more resilient fish, better able to cope with the physiological stresses of production cycle and environmental challenges encountered during production (Dong et al., 2023; Hundal et al., 2021).

In view of the above, this study aimed to evaluate different levels of soybean oil (SO) inclusion supplemented with the main LC-PUFAs as an alternative to FO, and to evaluate the effects of different 18:n-6/LC-PUFAs ratios on the fatty acid profile of liver and muscle tissue of juvenile *R. Canadum.*

**Material and Methods**

Fish handling followed the Ethics Committee's procedure protocols on the Use of Animals (CEUA nº 008/2017) of the University of Mogi das Cruzes (SP, Brazil). the animals were selected according to average body mass (10.70 ± 0.04 g) and transferred to 12 – 500 L tanks (15 animals per tank, in triplicate) in a recirculation aquaculture system (RAS) equipped with a UV, biological, and mechanical filters, pump, and water heater.

After producing the basal diet, the different experimental oils in FO, SO, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) were incorporated using the vacuum coating technique as previously described by Lamichhane et al., (2015), respecting the basic LC-PUFA nutritional requirements described for this species (Fraser et al., 2009; Trushenski et al., 2011; Araújo et al., 2019).

The control diet (FO) was composed by 8.8% of FO, while in the other experimental diets were included 8.8% of an oil blend, composed by coconut oil (CO) as a saturated fatty acid source, SO as C18 polyunsatured source, and LC-PUFA (ARA, EPA and DHA) concentrated sources in different concentrations as presented below: Diet 1 (D1) consisted of 2% CO, 6% SO, and 0.2%, 0.5%, and 0.1% of EPA, DHA, and ARA extracts, respectively. Diet 2 (D2) was prepared of 2% CO, 4.5% SO, and 0.7%, 1.2%, and 0.4% of EPA, DHA, and ARA extracts, respectively. Diet 3 (D3) was composed of 2% CO, 3% SO, and 1.0%, 2.0%, and 0.8% of EPA, DHA, and ARA extracts, respectively.

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During the trial, the experimental animals were hand-fed twice a day (08:00 and 17:00 h) until apparent satiation with the appropriate experimental diet for 8 weeks (56 days). At the end of the trial three animals from each tank were euthanized by benzocaine overdose, totaling nine animals per treatment, and aliquots of liver and muscle tissues were sampled and immediately frozen in liquid nitrogen, and posteriorly kept at -80°C.

The FA profile of liver and muscle was initiated by lipid extraction using dichloromethane andfurther methylated following the transmethylation method described by Parrish et al. (2014). The FA composition was analyzed as methyl esters (FAME) using gas chromatograph Scion 436 equipped with a flameionizer (FID) and CP 8410 auto-sampler. Data are presented as a percentage of total FAME based on peak area analyzed. All statistical tests were carried out using SigmaStat statistical software version 7.0 (Sigma Stat Software, CA, USA), using a significance level of 5% ($P<0.05$).

**Results and discussion**

In general, both in the liver and in muscle, saturated fatty acids (SFAs) were found in significantly lower proportions, while monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and long-chain polyunsaturated fatty acids (LC-PUFAs) showed higher deposition in all experimental groups, except in fish fed the FO diet, in which PUFA and LC-PUFA levels were lower. In muscle tissue, variations in total n-6 fatty acid levels were mainly determined by the pattern observed in 18:2n-6 concentrations, which increased in response to higher SO inclusion in the experimental diets (D1, D2, and D3, respectively). Similarly, significant differences were observed in ARA levels, with the lowest values in fish fed FO, followed by D1, D2, and D3. In the liver, the n-6 PUFA profile followed the same pattern observed in muscle, with higher levels in D1, intermediate in D2, and lower in D3, reflecting the different levels of SO inclusion in the experimental diets and, consequently, the variations in 18:2n-6 concentrations between groups.

Based on the composition of the experimental diets, a significantly lower deposition of SFAs and, conversely, a higher deposition of LC-PUFAs were observed in the tissues of the experimental animals. It is well established that, although there is a preferential order of fatty acids oxidation, aquatic organisms in general tend to preferentially oxidize nutrients that are present in excess in the diet, while conserving those supplied in limited amounts, a metabolic phenomenon known as fatty acid sparing effect (Turchini et al., 2011; Trushenski et al., 2013). The results observed in this study suggest a possible LC-PUFAs sparing effect, since compared to SFAs (primarily 12:0), DHA, EPA, and ARA were positively deposited, particularly in muscle tissue, even with relatively low percentages of these fatty acids in the experimental diets (especially D1 and D2).

**Conclusion**

In conclusion, SO supplemented with essential LC-PUFAs can effectively replace FO at different inclusion levels without compromising lipid metabolism in *R. canadum* juvenile. Moreover, the diet with the highest level of supplementation (D3) resulted in enhanced tissue deposition of LC-PUFAs, particularly in muscle.

# INFLUENCE OF ARA LEVELS ON PRODUCTIVE PERFORMANCE AND METABOLISM OF NILE TILAPIA (*Oreochromis niloticus)* JUVENILES MAINTAINED AT LOW TEMPERATURES

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**Introduction:**
*Oreochromis niloticus* is the main fish species produced in Brazil. However, the leading producers in Brazil were established in the south and southeast regions, where the autumn/winter temperatures are out of the optimum range for this species.

Previous studies showed that better growth and survival of fish at low temperatures were obtained by the incorporation of higher dietary levels of long-chain polyunsaturated fatty acids (LC-PUFA), especially docosahexaenoic acid (DHA) and arachidonic acid (ARA).

Current studies observed better production performance of tilapias at suboptimal temperatures when fed DHA-rich diets. However, the underlying mechanisms are unknown. In addition, despite the promising results obtained with other fish species, no studies evaluating the dietary ARA influence on the performance and metabolism of tilapia juveniles at low suboptimal temperatures have been carried out.

Thus, this study aimed to evaluate the efficiency of diets with different ARA levels on the productive performance of *O. niloticus* juveniles kept at suboptimal temperatures.

**Material and methods:**
All experimental procedures involving animals and biological tissues were evaluated and approved by the Ethics Committee on the Use of Animals of the University of Mogi das Cruzes (CEUA, Protocol No. 005/2024), in accordance with institutional and national guidelines.

Approximately 400 juvenile Nile tilapia (*Oreochromis niloticus*) were obtained from the Brumado Fish Farm (Mogi Mirim, São Paulo, Brazil) and transported to the facilities of the Ponte Nova Dam Fish Farm (Salesópolis, São Paulo, Brazil). Upon arrival, the fish were acclimated in a 4 $m^3$ tank for 15 days prior to the feeding trial.

After the acclimation period, fish were individually weighed and measured to establish the initial experimental batches. Animals were then randomly assigned to 15 tanks representing five dietary treatments (CTRL, ARA1, ARA2, ARA3, and DHA), in triplicate. The tanks consisted of multifilament hapas with a mesh size of 10 mm and a capacity of 2.4 $m^3$, installed within an earthen tank with a total volume of 600 $m^3$. Each experimental unit contained 20 fish with an initial mean body weight of 37.9 ± 0.8 g. The experiment was conducted during the winter season, with an average water temperature of 20.5 ± 1.2 °C in the Ponte Nova Fish Farm facilities. Water quality parameters, including dissolved oxygen (mean: 6.55 ± 0.7 mg $L^{-1}$) and water temperature, were measured daily throughout the experimental period. A basal diet was formulated according to the nutritional requirements for *Oreochromis niloticus* juveniles as proposed by Furuya (2012) and the NRC (2011).

From the basal formulation, five experimental diets were prepared to be isoproteic, isolipidic, and isoenergetic. In the control diet (CTRL), soybean oil was the sole lipid source. Diets supplemented with arachidonic acid (ARA) contained the following inclusion levels of concentrated oil (40% of ARA): ARA-1: 0.40%, ARA-2: 0.80%, and ARA-3: 1.20%, with a corresponding reduction in soybean oil to maintain similar lipid levels. A fifth diet was supplemented with 0.20% concentrated docosahexaenoic acid (DHA) oil, without ARA inclusion. The infusion of concentrated ARA oil, as well as other oils, was performed using a vacuum coating process as described by Lamichhane et al. (2015). The proximate composition of the experimental diets was analysed following the methodology of AOAC (2015).

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Prior to the feeding trial, ten fish from the initial stock were euthanized by eugenol overdose (0.1 g L□¹), and liver and muscle samples were immediately frozen in liquid nitrogen and stored at −80 °C until further processing. During the 56-day trial, fish were fed to apparent satiation twice daily (08:00 and 16:00 h) with their respective diets. At the end of the experiment, three fish from each tank (nine per treatment) were euthanized under the same protocol, and liver and muscle samples were collected for molecular, metabolic, and morphological analyses conducted at the Laboratory of Physiology and Nutrition of Aquatic Organisms (LAFINUTRI/UMC).

Lipid extraction from ingredients, diets, and tissues was performed using dichloromethane, followed by methylation according to the transmethylation method described by Parrish et al. (2014). Fatty acid composition was analysed as fatty acid methyl esters using a Scion 436 gas chromatograph equipped with a flame ionization detector (FID) and an auto-injector. Separation was performed on a CP Wax 52 CB capillary column (0.25 μm film thickness, 0.25 mm internal diameter, 30 m length). The oven temperature program was as follows: 170 °C for 1 min, ramped at 2.5 °C min□¹ to 240 °C, held for 5 min, totalling 31 min per run. Injector and detector temperatures were 250 °C and 260 °C, respectively. Fatty acids were identified by comparing retention times with a commercial FAME standard mix (Supelco, 37 components).

**Results and Discussion:**

After 56 days of the feeding trial, no significant differences were observed in the productive performance parameters among fish from the different dietary groups. These findings contrast with previous studies reporting a positive relationship between dietary ARA incorporation and improved growth and survival in commercial fish species such as *Rachycentron canadum* (Araujo et al., 2019), *Morone saxatilis* (Araujo et al., 2021), and *Seriola dorsalis* (Araujo et al., 2022).

As expected, and widely reported in other studies (Tocher, 2010; Glencross, 2009), the fatty acid profiles of liver and muscle tissues closely reflected the composition of the experimental diets. In general, ΣSFA was lower in all ARA- and DHA-supplemented groups compared with the control, with a significant reduction only in the ARA3 group for liver. Conversely, ΣPUFA deposition tended to be higher in supplemented groups, suggesting that fish fed the control diet had a lower capacity for PUFA retention, since similar dietary levels of this fatty acid class were provided in the experimental diets. ΣMUFA levels showed reductions in the ARA2 and ARA3 groups compared with the control in liver, but no significant variation among treatments in muscle.

EPA content was significantly higher in the DHA group compared with the control, but this effect was only observed in liver, with no differences detected in muscle. DHA levels, in turn, were significantly higher in the control group compared with the DHA-supplemented group in muscle, despite the latter receiving 1.54% purified DHA oil in the diet. ARA deposition patterns did not fully reflect the dietary inclusion levels. While the lowest concentrations were observed in the control group, the highest values were recorded in the ARA1 group for muscle and in the ARA3 group for liver.

**Conclusion:**

Dietary supplementation with ARA- and DHA-rich oils did not affect the productive performance of O. niloticus reared under suboptimal temperatures; however, it significantly altered the fatty acid composition of liver and muscle. Overall, supplemented diets decreased SFA deposition and increased PUFA retention. These results indicate selective retention and/or enhanced catabolism of LC-PUFA when provided in excess, supporting the hypothesis of tissue-specific regulation of fatty acid utilization. Such metabolic responses should be considered in tilapia feed formulation to improve nutritional quality and resilience to low-temperature rearing.

# BUILDING IMPACT THROUGH LEARNING: EDUCATING CHILDREN AND AQUACULTURE PROFESSIONALS ON ALDFG POLLUTION MITIGATION AND SUSTAINABLE AQUACULTURE

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**Introduction**

The aquaculture industry relies heavily on plastics and will likely continue as the world depends more on oceans for high-quality protein (Skirtun et al., 2022). Plastic fragments from aquaculture can break down into microplastics, harming the marine environment (Tian et al., 2022). FAO highlights that marine aquaculture installations are major sources of abandoned, lost, or discarded fishing gear (ALDFG), worsening marine plastic pollution (Macfadyen et al., 2009). However, education on best practices and plastic management can improve decision-making and help address the issue (Sandu et al., 2022). This study aims to emphasise the role of education in tackling plastic pollution from ALDFG, presenting the results research from educational activities in Greece.

**Materials and methods**

This research was conducted as part of the Healthy Seas Foundation's academic outreach project in Western Greece focusing on educating primary school students, university students, and academic staff about the impacts of marine plastic pollution from ALDFG and positive sustainable aquaculture practices. Educational programmes engaged 165 primary school students and their teachers in Astakos, Nafpaktos, and Amphilochia. These activities incorporated outdoor learning to enhance engagement and knowledge retention. The partnering European Outdoor Education Hub contributed by integrating experiential outdoor education methodologies. Activities included hands-on engagement with coastal environments, observation-based learning, and experimental exercises to help students grasp the real-world impact of ALDFG pollution. Experiential field sessions on the school grounds and the local seashore encouraged direct participation, fostering a deeper connection to marine conservation efforts. Adult education involved 8 university faculties from the Universities of Patras and Ioannina, with 205 students and staff, covering topics like plastic pollution from ALDFG and waste management best practices. A webinar expanded the reach globally. To assess the impact, questionnaires were used to measure changes in knowledge and perceptions, focusing on plastic pollution awareness and sustainable aquaculture practices.

**Results**

The in-person activities reached 370 people, including 205 university students and staff and 165 primary school students and teachers. The webinar engaged 107 additional participants. The questionnaire analysis revealed that while most participants were initially unfamiliar with ALDFG, 99.5% reported increased understanding after the seminar. 55% felt their knowledge had greatly improved. Audiovisual materials helped 64.6% better grasp the content, and 20% showed interest in further studying sustainable aquaculture practices, indicating the potential of education to promote sustainability. Regarding the results of the primary school fieldwork, qualitative feedback from teachers and students indicated that outdoor, experiential learning played a key role in improving engagement and knowledge retention. Educators, through Likert-Scale and open-ended questions noted that students were more receptive to conservation messaging when involved in hands-on activities in natural environments. This aligns with broader research on outdoor education's effectiveness in environmental literacy and behavioral change.

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**Discussion**

Education and training of the aquaculture workforce can contribute to enhancing the sustainability of the sector and empowering coastal communities. (Berkowitz et al., 2022). Similarly, educating children is equally important, as it lays the foundation for the next generation to become environmentally conscious and actively contribute to the protection of marine ecosystems (Freitas et al., 2023). The integration of outdoor education as part of the activities further amplified this impact, reinforcing the importance of immersive, place-based learning in fostering pro-environmental attitudes (Sobel 2005). Beyond traditional classroom settings, experiential learning in natural environments has been shown to enhance ecological awareness and long-term behavioral change, making it a crucial component in sustainability education. Adults preferred visual materials to understand plastic pollution, supporting studies on audiovisual learning effectiveness. (Kolandai-Matchett et al., 2021). In conclusion, modern education methods are key to raising awareness of environmental issues.

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# GONADOTROPIN PLASMID GENE THERAPY TRIGGERS GAMETOGENESIS IN MALE AND FEMALE EUROPEAN EEL (*Anguilla anguilla*)

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## Introduction

The European eel is a species of high economic impact for both commercial fisheries and aquaculture in Europe. However, the eel does not reproduce naturally in captivity. Hormonal induction with traditional methods has been applied to address reproduction problems, including weekly administration of human chorionic gonadotropin (hCG) in males (Pérez et al., 2000) and salmon or carp pituitary gland extracts, followed by a final dose of progestogen to induce spawning, in females (Gallego et al., 2012). Despite their effectiveness, these methods are time-consuming and expensive. An alternative approach to induce gametogenesis in captivity can be the use of gene therapy using recombinant gonadotropin plasmids (Mazón et al., 2013). The present study tested the potential of this technique in male and female European eels, by transferring and overexpressing homologous gonadotropin genes.

## Materials and methods

Two independent experimental trials were conducted on immature European eel males maintained in seawater at 20 ºC: Experiment 1: Fish with an average weight of 142.64 ± 2.84 g were subjected to three treatments: i) fish injected with empty pCDNA3 plasmid (control), ii) fish injected with eel single-chain follicle-stimulating hormone (scFsh), and iii) fish injected with eel scFsh + eel single-chain luteinizing hormone (scLh). To monitor injection effectiveness, all groups were injected with 15 µg of sea bass (*Dicentrarchus labrax*) scFsh which can be monitored in plasma and has no functional effect in eel. Injections were performed in weeks 1, 3 and 6 in duplicate (reinjecting after 3 days each time), and total duration of the experiment was 9 weeks.

Experiment 2: Fish with an average weight of 103.54 ± 1.29 g were subjected to three treatments: i) fish injected weekly with hCG (positive control); ii) fish injected every three weeks with empty pCDNA3 plasmid (negative control), and iii) fish injected every three weeks with the combination of eel scFsh at a constant dose and increasing doses of eel scLh plasmids. Total duration of this experiment was 15 weeks.

One experimental trial was conducted on immature European eel females maintained in seawater at 20 ºC. Fish with an average weight of 756.95 ± 12.74 g were subjected to three different treatments similar to experiment 1 for males.

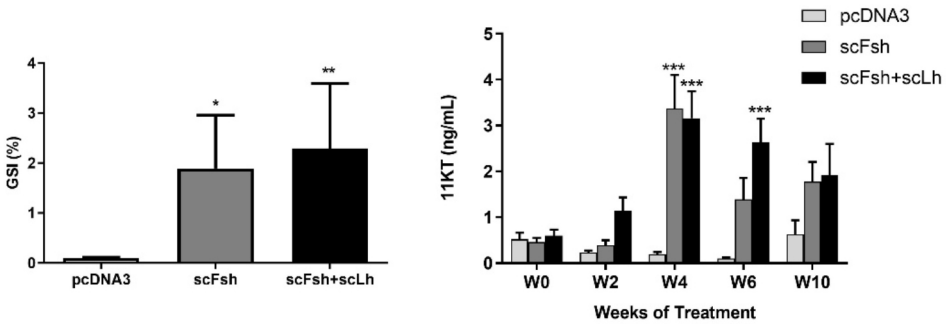


**Fig. 1.** Gonadosomatic index (left) and plasma 11 KT levels (right) in male European eels in experiment 1. Data are shown as mean ± SEM (n = 5, 6, and 8 per sampling and treatment). Asterisk indicates significant differences between treatments at the end of the trial.

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To evaluate maturation progression, blood samples were taken at the times of injections for steroid analysis, and biometric parameters (eye index, fin colour and weight) were recorded. Additionally, at the end of each experiment, the fish were sacrificed, the gonadosomatic index (GSI) was determined and tissue samples were preserved for histological analysis.

**Results**

In experiment 1, treated male European eels showed significantly higher GSI and plasma steroid levels compared to control groups (Fig. 1). These findings indicate that gene therapy with homologous gonadotropin effectively stimulates spermatogenesis. However, there was some variability among individuals, and only a subset of the treated eels progressed to spermiation.

To improve these results, the treatment protocol was optimized in experiment 2, and a positive control using traditional hCG treatment was included for comparative purposes. Results showed that GSI and steroid levels in plasmid-treated group were similar to the hCG group and significantly different from the control one, indicating enhanced effectiveness of the revised gene therapy protocol. Similarly, biometric parameters such as eye index and fin coloration increased over time in the treated groups, whereas they remained unchanged in the control group (pCDNA3). In addition, nearly all the treated specimens achieved spermiation by the end of the trial.

In the female experiment, treatment with European eel recombinant gonadotropin plasmids led to significant increases in biometric parameters, oogenesis progression, and sex steroid levels compared to the control group, highlighting the potential of gene therapy as an alternative method to induce reproduction of European eel females in captivity.

In conclusion, compared to conventional hormonal treatments, the results obtained in our study show that gonadotropin plasmid-based gene therapy offers a promising, cost-effective, and efficient alternative for overcoming reproductive dysfunctions in European eel aquaculture.

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# A NATURAL MICROENCAPSULATED ANTIOXIDANT TO MITIGATE DIETARY MICROPLASTICS ACCUMULATION AND ADVERSE EFFECTS IN JUVENILE EUROPEAN SEABASS (*Dicentrarchus labrax*) AQUACULTURE

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## Introduction

The aquaculture sector is susceptible to the contamination from microplastics (MP) which mainly derive from environmental pollution, the use of plastic tools, and fish feeds, whether in freshwater or marine farming. Although fish possess the ability to differentiate suspended MP from edible particles, the inherent contamination of aquafeeds presents a less detectable exposure route (Siddique et al., 2023). This results in increased MP ingestion by farmed fish, thereby posing potential health risks to the fish themselves and raising concerns for consumer safety. In fact, dietary MP smaller than 20 μm can be absorbed in the fish intestine, leading to a consequent translocation to other tissues, among which liver acts as a retaining organ (Cattaneo et al., 2024). This limits the accumulation in other sites but causes the triggering of oxidative stress. In this context, the dietary supplementation of natural antioxidants, like astaxanthin (AX), could strengthen the intrinsic defence mechanisms of fish, contributing to mitigate the MP-induced oxidative stress. Particularly, natural AX has a higher antioxidant efficacy compared to its synthetic counterpart. However, natural AX is characterized by a rapid degradation due to its low stability, particularly under industrial processing conditions. To address this limitation, microencapsulation techniques have emerged as promising strategies to enhance the stability of the AX molecule and improve its bioavailability and delivery through aquafeeds. Additionally, the microcapsules' wall matrix can have a crucial effect in preventing the MP absorption at fish intestinal level. In fact, starch, one of the most frequently preferred components for microcapsules preparation, has been demonstrated to aggregate MP in both suspended water and inside fish gut. Within this context, the present study aimed to: (i) implement fish feeds with natural AX included in microcapsules made of an organic starch-based wall matrix able to preserve it and, at the same time, to exert a crucial role in limiting the MP absorption at fish intestinal level; (ii) testing the experimental diets on European seabass (*Dicentrarchus labrax*) juveniles over a 60-day feeding trial; (iii) investigating the MP assimilation and accumulation in different fish tissues along with their overall health status.

## Materials and Methods

Starting from a control fluorescent MP-free diet (named C), a MP diet, was prepared by adding purchased fluorescent microbeads (range size 1-5 μm) to the control mixture (50 mg/kg feed). Then, the C+AX and MP+AX diets were prepared by adding 7 g/kg feed of AX microcapsules (corresponding to 175 mg/kg of AX) to the C or MP diets, respectively. Five hundred and forty European seabass juveniles were then divided in six experimental group (in triplicate) and were fed as follows: (i) C group: C diet for 60 days; (ii) C/C+AX group: C diet for the first 30 days and C+AX diet for the following 30 days; (iii) C+AX group: C+AX diet for 60 days; (iv) MP group: MP diet for 60 days; (v) MP/MP+AX group: MP diet for the first 30 days and MP+AX diet for the following 30 days; (vi) MP+AX group: MP+AX diet for 60 days. Growth indexes were calculated by weighting all the fish at the end of the trial. MP were localized with confocal microscopy in fish intestine, liver, blood, muscle and adipose tissue, whilst they were quantified on filters through epifluorescence microscopy after digesting the above-mentioned tissues with a KOH solution. Fish welfare was assessed with histological and molecular (real-time PCR on markers involved in immune and oxidative stress response) analysis on liver and intestine samples. Finally, to assess their potential role in MP aggregation, whole microcapsules (empty or filled with AX), and starch (as main microcapsules' component) were tested in an *in vitro* experiment.

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**Results and Discussion**

The different dietary treatments did not affect fish survival rate and growth performance and did not cause pathological alterations or signs of inflammation in the intestine as evidenced by both the histological indexes and the relative expression of immune response markers (*il1b*, *il10*, and *tnfa*). However, the dietary fluorescent MP microbeads were absorbed at intestinal level, leading to a consequent translocation and accumulation in the liver which caused, in fish from the MP group, a *sod1*, *sod2*, and *cat* upregulation. Interestingly, the dietary implementation of AX microcapsules led to a mitigation in the expression of oxidative stress markers, particularly in the MP+AX group where it reached levels comparable to those observed in all the controls. Additionally, the microcapsules used in the present study played a significant role in limiting the intestinal MP absorption. In fact, fish from MP+AX group revealed a significantly lower intestinal absorption of fluorescent microbeads, leading to a consequently reduced accumulation in all the organs analysed compared to those from MP group. Of particular interest was the absence in the muscular tissue. These results were supported by: (i) an *in vitro* coagulation test which demonstrated that the microcapsules (empty or filled with AX) and the starch, tested at the same concentration used in the experimental diets, effectively trapped the fluorescent microbeads, forming MP aggregates too large to be absorbed at the intestinal level; (ii) the number of fluorescent microbeads detected in the fish faeces samples which was significantly higher in both the MP/MP+AX and MP+AX groups compared to the MP one. These results can be of great interest for the aquaculture sector in light to promote fish welfare and quality product. Further studies are necessary to depict a more comprehensive overview of the effectiveness of this technology in relation to the wide variability of conditions within the aquaculture sector and the types of polymers that can be ingested by the fish.

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# DEVELOPMENT OF AN AUTOMATED HATCHING SYSTEM PROTOTYPE FOR EARLY DEVELOPING RAINBOW TROUT (*Oncorhynchus mykiss*) EMBRYOS TO REDUCE FOOD LOSSES

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## Introduction

Rainbow trout (*Oncorhynchus* mykiss) is the main freshwater species farmed at European level, both in terms of volume and value. In fact, almost all rainbow trout on the EU market comes from aquaculture since this species does not naturally reproduce in most of European aquatic basins, in both fresh and saltwater. The main EU member states farming this species are France, Italy and Denmark, accounting for 56 % of EU production. Monoculture is the most common rainbow trout farming, and intensive systems are considered necessary in most situations to make the operation economically attractive. The rainbow trout production cycle can be divided into different phases, each characterized by important critical aspects that must be considered to ensure high productivity rates and high-quality standards. The knowledge behind most of these phases has been deeply consolidated over the years, allowing farmers to minimize loss in the supply chain. The most critical step is still represented by the early embryo development, lasting from fertilization to the achievement of the eyed egg stage. Fertilized eggs are commonly incubated in vertical flow incubators, left undisturbed until the achievement of the eyed stage. Developing embryos are subjected to visual inspections and to the eventual manual removal of dead ones, followed by the use of chemicals for disinfection to prevent the spreading of infections. In fact, during this incubation stage, high mortality rates are caused by infections by ubiquitous Oomycete species (*Saprolegnia* sp.) and other pathogens that led, in some cases, to the loss of entire batches of embryos. Since oomycetes can colonize the surface of dead embryos while suffocating the surrounding living ones, a quick pathogen detection is necessary. However, if the infection is extensively spread and most embryos results infected after the visual inspection, the entire batch of embryos contained in the vertical incubator (infected and alive) is generally discarded. Within this context, the present research aimed to develop and validate a technological automatic vertical incubator prototype, equipped with a set of multi-spectral cameras and specific tools, able to gently lift the embryos and to quickly recognize and remove the non-developing or infected ones, in light to reduce possible food losses.

## Materials and Methods

In a preliminary stage, 50 batches of rainbow trout embryos, obtained from an Italian supplier, were analysed with XIMEA multi-spectral cameras. Particularly, embryos of each batch, both good (orange) and bad (white), were disposed on a single layer, and cameras acquisitions were performed in the visible (VIS) and near infrared (NIR) spectrum. The same procedure was conducted covering the single layer of embryos with water to simulate the state of immersion. The collected images (3 images per batch with and without water) were labelled by domain experts and then used to train a deep learning model able to detect eggs and assign label based on reflectance data. This preliminary research was necessary to identify (spectral) bands of interest.

A prototype of the vertical incubator was then developed integrating computer vision technologies and environmental sensors and tested in farm conditions over a seven-day period. Particularly, high-resolution Basler USB 3.0 cameras (constituting the vision system) acquired images in both RGB and grayscale format that were acquired by a computer equipped with a GPU that reduces the inference time of a deep learning model to detect healthy and diseased embryos. Inside the vertical incubator, a 45° tilted cochlea was placed to gently lift the embryos from the bottom of the system to the sorting area where multi-spectral cameras can discriminate them. The prototype was integrated with a Pacific system equipped with a series of probes and sensors to monitor water conditions (pH, temperature, dissolved oxygen, and $CO_2$) and ensure the correct functioning of the system. Finally, a water flow meter ensured that embryos were correctly lifted and transported by the cochlea without excessive turbulence or stagnation. The embryos were exposed daily to one complete transit in the cochlea and to the camera view (for selection). After the experimental period (7 days), the embryos were placed in traditional vertical hatchery systems until the hatching. Their hatching rate was then compared with that of part of the same batch embryos, reared in conventional hatchery systems.

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**Results and Discussion**

The prototype efficiently lifted the embryos without affecting their integrity and the cameras discriminated them with an overall accuracy of 94%. The RGB colour images allowed for more detailed analysis of the surface, while grayscale images allowed for greater precision in detecting changes in intensity that could indicate anomalies with the capability to filter specific band of interest. At the end of the experimental period, the hatching rate of the embryos reared in the prototype did not show significant differences compared to those maintained in conventional hatchery systems. This first-phase prototype completely automated the whole embryo selection process, reducing the need for manual intervention and increasing the selection accuracy. Furthermore, it was possible to continuously monitor both the state of the embryos and the water parameters, ensuring the maintenance of optimal environmental conditions for their development. The integration of high-quality cameras, powerful deep learning models and the environmental monitoring made the prototype extremely effective and precise, optimizing the success of the incubation process. In the next phase, the prototype will be further improved with a device able to remove bad eggs according to cameras-derived data.

Despite a not immediate large-scale application in a farming *scenario*, this device could improve up to 5% the number of embryos that achieve the eyed stage (less delicate), thus improving the whole productivity of rainbow trout farms.

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# DETERMINATION OF FOOD LOSS CAUSES WITHIN MEDITERRANEAN MUSSELS (*Mytilus galloprovincialis*) PRODUCTIVE CYCLE: A CASE STUDY IN THE ADRIATIC SEA

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## Introduction

The farming of Mediterranean mussel (*Mytilus galloprovincialis*) is a crucial sector for Italian aquaculture, accounting for the 45.6% of the national seafood production. Particularly, the western Adriatic Sea offers favourable environmental conditions for the extensive farming of this species. In this area, Mediterranean mussels are exclusively cultured in offshore waters using the long-line system which comprises a horizontal backbone from which multiple ropes colonized by mussels are suspended. Despite its simplicity, this farming technique represents a low-waste, cost-effective, and sustainable seafood production methods, while providing numerous ecosystem services including: (i) improvement of water quality due to the mussels' filtration activity; (ii) increasing the substrate complexity in sandy bottom areas, promoting biodiversity; (iii) safeguarding the coastline habitat preventing its erosion; (iv) reducing $CO_2$ emissions acting as a carbon sink deposited in their shells. This specific case study was performed in the Adriatic Sea, following a productive cycle that started in June 2024. The initial phase was the collection of seeds that derived either from natural settlement on farm infrastructure (ropes, cables, and buoys) or from routine cleaning operations residual. During the on-growing phase, mussels were subjected to seasonal changes, natural climatic conditions, seawater features, and eventual presence of predators that can variably affect the productive cycle. In addition, human intervention during the harvesting processes can introduce different stressors that can impair mussels' overall health and marketability. Particularly, a first harvest occurred in February 2025 during which mussels under market size were collected to be shipped to different marine farming areas for a final relocation phase that, due to the different seawater features, can enhance organoleptic properties and support the valorisation of regionally branded mussels. In this first harvesting phase, no cleaning procedure is allowed, and mussels socks are stacked on pallets and wrapped with cling film to ensure containment and to reduce desiccation during transport on refrigerated trucks. A second harvest, that occurred in May 2025, is intended for the local distribution and involves mussels at market size. At this stage, they were harvested and subjected to a cleaning procedure that, although necessary for commercial presentation, can damage shells and byssal threads, compromising mussel survival. Within this context, the aim of the present study was to monitor the productive cycle of a Mediterranean mussel farm located in the mid-western Adriatic Sea to investigate how the harvesting procedures can affect mussels' health status and marketability.

## Materials and Methods

The complete production cycle and the harvesting steps (in February for shipment and in May for local market) were monitored at a commercial farm named Sena Gallica Soc. Cooperativa (Senigallia, AN, Italy; 43°43′N, 13°13′E). During the first harvest, 36 socks (1 m each) were collected prior to the shipment and transported in seawater to the laboratory. A significant number of specimens were immediately sampled from 9 one-meter samples ($t_0$). Then, to replicate the transport conditions, the remaining 27 socks were stacked on three pallets (9 socks each), composing three layers (3 socks each) defined as follows: bottom (BOT), intermediate (INT), and top (TOP). The stacked pallets were then maintained in a climatic chamber set at 7 ± 1 °C for 17 hours, simulating the duration and temperature of typical refrigerated transport. At the end of the 17 hour-period ($t_1$), mussels were sampled from the socks located in each layer (BOT, INT, TOP) on each pallet to evaluate changes, resulting from the transport process, in marketable features and health status investigated through histological (application of histopathological indexes to determine the status of the organ) and molecular (real-time PCR on markers involved in general and oxidative stress response) analysis. During the second harvest, 9 socks were subjected to the cleaning procedure through the automatic machine (company: Cocci Luciano srl) that allowed the separation of all the mussels' categories: (i) cleaned mussels to be sold; (ii) mussel seed; (iii) dead mussels under market size; (iv) dead mussels at market size; (v) mussels damaged by the cleaning procedure. Finally, the percentage incidence of each category (based on their total weight) was calculated.

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**Results and Discussion**

Considering the first harvest, the simulated transport conditions slightly affected the health status of the mussels stacked in the lowest layer of the stack. In fact, at $t_1$, mussels from the BOT layers highlighted a significantly higher relative expression of markers involved in the oxidative stress response (*sod1* and *cat*) in the gills. This result was supported by a higher incidence of histopathological indexes in gills of mussels located in the BOT layers, including intracellular inclusion of lipofuscin-like substances, epithelium detachment or loss, and lamellar deformations. Differently, the health status of the digestive gland was not altered by the transport conditions as demonstrated by the absence of differences in both histological and molecular analyses. These outcomes can be explained by the higher susceptibility of mussels' gills to oxidative stress caused by environmental fluctuations. In fact, it should be considered that several factors that may occur during transport, including drastic temperature reduction, excessive air exposure or prolonged emersion, and hypoxia conditions due to the packaging method used, can contribute to trigger an oxidative stress response, possibly impairing: (i) survival rate for part of the specimens that are put back in the sea to complete the productive cycle; (ii) the nutritional quality features for the small fraction of specimens that are sold, immediately after shipment, to local market. Considering the second harvest, the percentage of mussels ready to be sold derived from the cleaning procedure accounted for the 77.17 ± 3.63 % of the total sock' weight. The second most represented fraction was the mussels seed that accounted for 14.85 ± 2.87 % of the total sock' weight. The mussels seed represents an interesting fraction: in fact, despite they don't constitute a sellable product, seeds are recovered by the farmer and are used as starting point for the following productive cycle. The dead mussels under market size (0.85 ± 0.12 %) constituted normal production losses since were specimens that died for natural reasons (environmental parameters or presence of predators) during the previous months. The real food loss sources were represented by the dead mussel harvested at market size (2.25 ± 0.53 %) and by mussels irreparably damaged by the cleaning procedure (3.27 ± 0.67 %) which affected the total farm production for 0.36 and 0.68 %, respectively. Differently, slightly damaged mussels, which accounted for 1.62 ± 0.29 % of the total sock' weight, were characterized by minor shell fractures that did not impair the survival from harvesting to the consumer. The discrimination of broken mussels according to the degree of the damage allowed to not overestimate the food loss sources introduced by the automatic cleaning machine. These findings highlighted the major food loss sources that affected the productive cycle analyzed within the present case study and could represent a starting point to be compared with other farms and to optimize the supply chain, minimizing losses.

**Funding**

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# DEVELOPMENT OF A MICROBIOME-BASED EARLY DETECTION SYSTEM FOR STANDARDISED QUALITY ASSESSMENT IN PIKEPERCH *Sander lucioperca* LARVAE

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**Introduction**

The rearing of new aquaculture-relevant species often faces challenges due to limited knowledge about nutritional requirements and other key parameters, frequently resulting in morphological deformities in embryos and larvae. Among the major bottlenecks in the successful cultivation of pikeperch is the low survival rate of larvae, which is often accompanied by a high incidence of malformations (Policar et al.2019; Colcen et al.2020). One of the suspected contributors to these deformities is the microbial environment, particularly the presence of pathogenic or toxin-producing bacteria. Stress sensitivity during critical development phases—especially during the transition to dry feed—can further exacerbate the impact of bacterial pathogens which may lead to the growth of harmful bacteria that produce toxins capable of inducing developmental abnormalities. To better understand and prevent such deformities, continuous monitoring of microbial community composition in the rearing water is essential. A simplified and largely automated system for detecting and quantifying microbial contamination—especially focusing on toxin-producing bacteria—would represent a valuable tool for hatcheries. It would support timely interventions, reduce economic losses, and contribute to higher larval quality and survival. Therefore, the aim of the study was to analyse and quantify specific pathogenic bacteria within the microbial community in the rearing water of pikeperch (*Sander lucioperca*) larvae. Key bacterial species were identified using qPCR and molecular biology techniques, and corresponding marker genes were developed to enable their rapid detection. In addition, the study investigated the relationship between microbial community composition, water quality, and larval deformities during early development stages. The findings contribute to the advancement of sustainable aquaculture practices and support the development of an automated QualiScore system for the early prediction of larval batch quality.

**Material and Methods**

Water samples were collected from a commercial pikeperch (*Sander lucioperca*) hatchery throughout the larval rearing phase to monitor microbial dynamics, starting from tank water without larvae ($t_0$) and continuing through key developmental stages: 4, 9, 12, 23, 30, 39, and 56 days post-hatch (dph), corresponding to major physiological changes such as mouth opening, swim bladder development, transition to dry feed, onset of cannibalism, disease occurrence, and size differentiation. Samples were taken from three different rearing tanks and a biofilter system, with six replicates collected per tank per time point (n = 168). At 56 dph, additional samples (n = 18) were collected from a single tank by separating larvae into small, medium, and large size groups. Bacterial DNA was extracted from 189 samples, and DNA concentration and purity were measured using a Nanodrop spectrophotometer. Microbial community composition was analyzed using 16S rRNA gene amplicon sequencing, and key bacterial taxa were identified. To enable rapid detection of pathogenic and toxin-producing bacterial strains, specific primers for marker genes were developed and quantified using qPCR.

**Results and Discussion**

Preliminary results showed that microbial analysis of pikeperch larvae revealed shifts in bacterial composition over time and differences by size at 56 dph, highlighting the emergence of Flavobacterium infections at later growth stages. Flavobacterium and Pseudomonas increased in abundance from 12 dph onwards, with early stages showing more diverse profiles. By 23 dph, these genera dominated, likely due to changes in environmental conditions or feeding. The shift towards Flavobacterium suggests a potential risk for emerging infections at later stages of growth.

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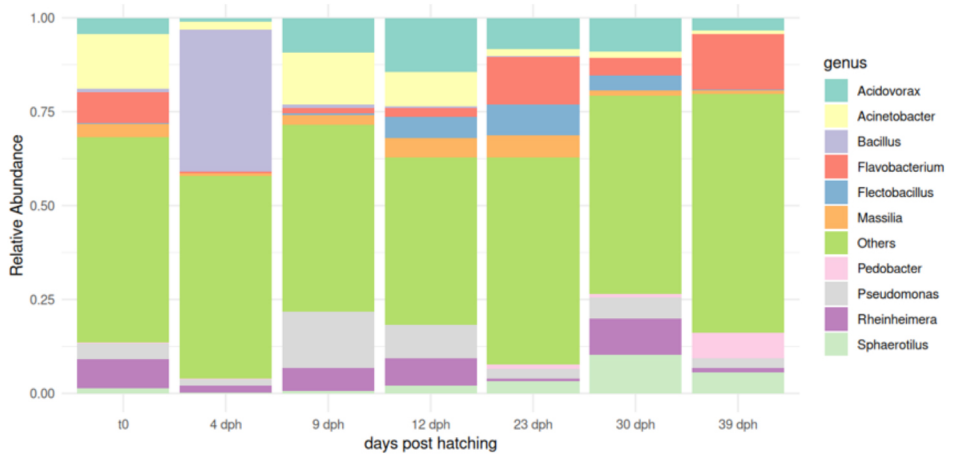


Fig.1. Microbial composition of pike perch rearing water over time.

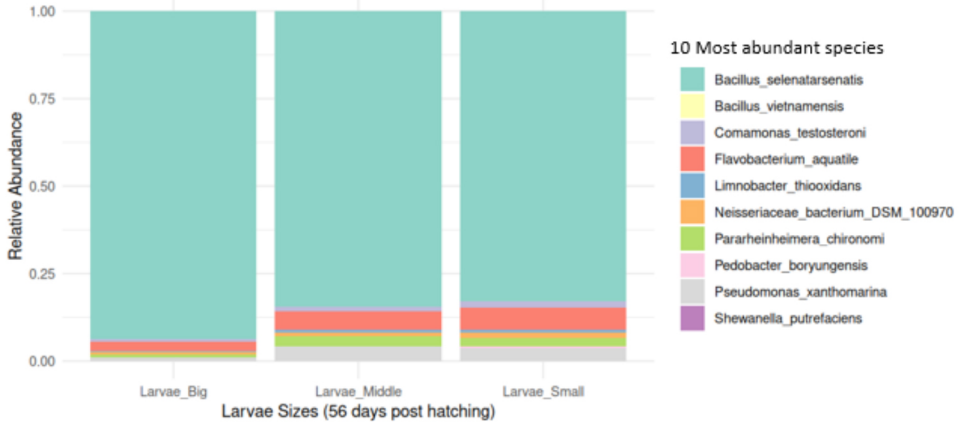


Fig.2. Microbial composition of pike perch larvae during rearing.

At 56 dph, larvae grouped by size showed a microbiome dominated by Bacillus species, with smaller larvae having higher levels of harmful genera like Shewanella and Pseudomonas, indicating greater vulnerability to pathogens. However, the high abundances of Pedobacter and Bacillus species suggest a stable microbial community at different developmental stages, which may contribute to disease prevention and better larval health. These findings emphasize the importance of monitoring entire microbial community composition, particularly emerging pathogens like Flavobacterium, to improve larval health and reduce mortality in hatcheries. The presence of beneficial bacteria like Pedobacter and Bacillus may also help prevent diseases in fish.

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# ASSESSING THE IMPACT OF NANOBUBBLES AND ANTIBACTERIAL COATINGS ON LIVE FISH TRANSPORT SYSTEMS

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## Introduction

The aquaculture industry is expanding rapidly, with logistics accounting for 25% or even more of production costs. Improving transport efficiency while ensuring fish welfare and reducing environmental impact is critical for the sustainable growth of aquaculture. Live fish transport faces challenges such as hypoxia, poor water quality, and bacterial contamination, which can result in significant animal losses. Recent advancements in nanotechnology, specifically nanobubble oxygenation and antibacterial coatings, offer promising solutions to address these issues. Studies have demonstrated that hypoxia is a major cause of stress and mortality in transported fish (Berka, 1986; Gomes et al., 2003; Ren et al., 2022). Nanobubble oxygenators have been shown to improve oxygen levels in controlled environments, extending fish survival during transport (Wang & Wang, 2023). Additionally, antibacterial coatings can reduce microbial contamination in transport water (Dube, 2024). Therefore, the aim of the study was to develop an improved live-fish transport system that enhances welfare, lowers bacterial loads, and reduces costs and emissions.

Two experimental approaches were conducted: (a) testing antimicrobial coatings on tank surfaces to reduce pathogens like *Flavobacterium psychrophilum*, and (b) applying nanobubble oxygenation to improve live transport of rainbow trout. Laboratory trials used a bioreactor to assess bacterial growth and biofilm formation via microscopy and flow cytometry. In parallel, field tests evaluated nanobubble technology for enhancing oxygen supply and fish welfare. The combined aim is to support more sustainable and health-conscious transport systems for rainbow trout.

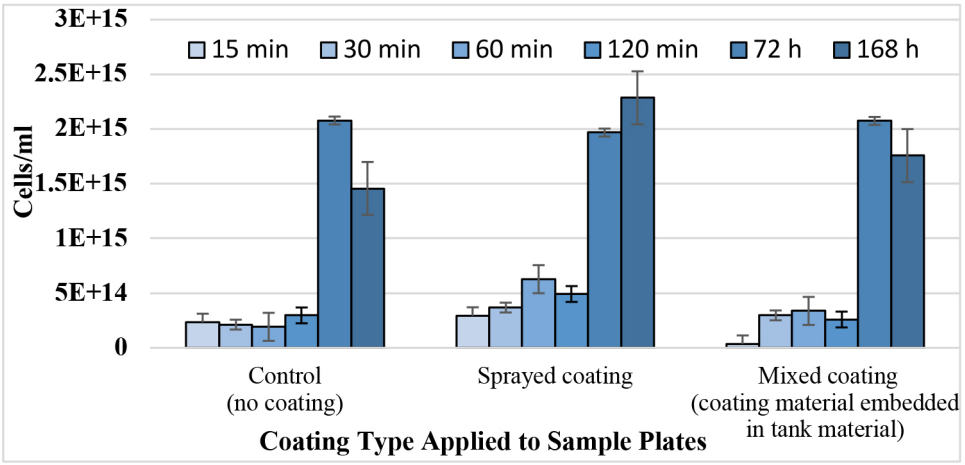


Fig. 1. Attachment of the test bacterial strain *Flavobacterium psychrophilum* over time on sample plates with different antibacterial coatings.

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**Material and Methods**

a) *Coating experiment*: In this experiment, we tested the antibacterial effectiveness of different coatings applied to sample plates—sections of tank wall material specifically cut and prepared for laboratory testing. Three coating types were investigated: (1) Control (no coating), (2) Sprayed coating, and (3) Mixed coating (coating material embedded directly into raw tank wall material). To assess bacterial attachment and biofilm formation, we first cultured the bacterium *Flavobacterium psychrophilum*, a pathogen commonly associated with disease outbreaks in rainbow trout (Oncorhynchus mykiss), our test species. The coated and control plates were then placed into a bioreactor system simulating aquaculture conditions. This system consisted of eight rotating sticks, each randomly fitted with one of the three coating types (four replicates per group). The coated plates were exposed to bacterial growth medium (TYES) for defined time intervals: 15 and 30 minutes, and 1, 2, 72, and 168 hours. After each time point, samples were taken, and the plates were stained using the DAPI method to visualize attached bacterial cells. Bacterial attachment and biofilm formation were then analyzed via fluorescence microscopy and flow cytometry to quantify cell numbers on each coating type.

b) *Nanobubble Experiment*: To evaluate the effect of nanobubble aeration on water quality during live fish transport, we conducted a transport simulation experiment using six tanks. Three tanks were equipped with a standard aeration system (control group), and three tanks used a nanobubble generator (experimental group). Each group included three replicates. Nanobubbles are extremely small gas bubbles, typically 100–200 nm in diameter, that can remain suspended in liquid for extended periods due to their neutral buoyancy and stability. These properties allow nanobubbles to deliver oxygen more efficiently than conventional aeration methods, potentially improving dissolved oxygen levels in closed transport systems. All tanks were filled with water directly pumped from rainbow trout rearing ponds to replicate real-world transport conditions. Throughout the experiment, key water quality parameters—including dissolved oxygen, ammonia, nitrite, nitrate, and pH—were continuously monitored. Water samples were collected at both the start and end of the experiment to assess bacterial load. These samples were analyzed using DAPI staining to count bacterial cells and via DNA extraction for later molecular analysis of the microbial community structure.

**Results and Discussion**

Attachment of the test bacterial strain *Flavobacterium psychrophilum* showed no significant differences between coating types and increased over time, with the highest cell counts observed at 72 and 168 hours. While some differences in cell counts were observed between the coatings, no consistent pattern or clear advantage of one coating over another could be identified (Fig.1). In the meantime, a new set of coated plates has been prepared for field testing at commercial trout farm. The plates will be submerged in pond water to evaluate coating performance under real environmental conditions. Further results from the coatings experiments are pending to confirm these initial findings and to evaluate potential trends and performance in live fish transport. In the nanobubble experiment, oxygen levels reached 200% within 15 minutes and exceeded the measurement limit after 60 minutes.

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# AN IMAGE SYNTHESIS APPROACH TO IMPROVE SALMON LOUSE LARVAE (*Lepeophtheirus salmonis*) DETECTION IN CHALLENGING SEAWATER ENVIRONMENTS

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**Introduction**

Salmon lice (*Lepeophtheirus salmonis*) pose a serious ecological and economic threat to both farmed and wild salmon. Detection of salmon louse larvae—especially during pre-infective and infective stages—can be crucial for timely intervention. While polymerase chain reaction (PCR) offers high accuracy, it is costly and to date impractical for continuous real-time monitoring. Machine learning offers a promising alternative, but current models often fail in real-world conditions due to limited annotated data and training on simplified laboratory environments. Robust detection systems must perform reliably across changing water conditions and plankton communities, which requires diverse datasets based on samples collected in the field. This remains a major challenge to achieving generalizable and scalable solutions.

**Materials and methods**

To address these challenges, we propose a synthetic data generation framework based on the Segment Anything Model (SAM) tailored for the detection of salmon louse larvae across multiple developmental stages in real-world seawater conditions (Fig. 1). A total of 54 orthogonal experiments were conducted to optimize the parameter configuration, leading to the creation of a high-quality synthetic dataset comprising 120,864 images. To fully exploit the synthetic data and improve detection performance, we evaluated models from the YOLO series alongside the transformer-based RT-DETR-L model. Four distinct training strategies were systematically tested: (1) Baseline - trained solely on the original training set; (2) Synthetic-Only - trained only on the synthetic dataset; (3) Fine-tuning - pretrained on the optimal synthetic set, then fine-tuned on the original data; (4) Combined - trained on a merged set of synthetic and original data. The final model was further validated on real-world seawater samples to assess its generalizability and robustness.

**Results and Discussion**

Our experiments demonstrated that pretraining on synthetic data—followed by fine-tuning or combined training—consistently enhanced model performance across multiple evaluation metrics (Fig 2). For instance, the YOLOv8n model's F1-score improved from 70.6% to 87.7%, representing a substantial relative gain of 24.22%. In real-world seawater tests, models trained with synthetic data achieved an 11.0% increase in recall, reaching 87.0%, compared to models trained solely on original data. Notably, these models also outperformed experienced human biologists, further validating the effectiveness and practical applicability of the synthetic data generation approach.

Our synthetic data approach offers a flexible and scalable solution for marine parasite detection, with strong potential for transferability across regions, seasons, and species. Although initially developed using local samples, the model effectively captures broad plankton diversity. By integrating representative samples and synthetic data from new environments, it can rapidly adapt and improve based on real-world inputs. This adaptability enhances model robustness and enables large-scale, real-time monitoring of salmon lice and other marine organisms.

**Conclusion**

This study presents a scalable and effective approach for monitoring salmon louse larvae in complex marine environments. By leveraging synthetic data, it overcomes key challenges in data collection and annotation, enabling robust model performance under real-world conditions and rapid adaptation to new environments. The method outperforms expert detection, highlighting its strong potential for large-scale application in seawater surveillance and enhancing health management of both farmed and wild salmon populations.

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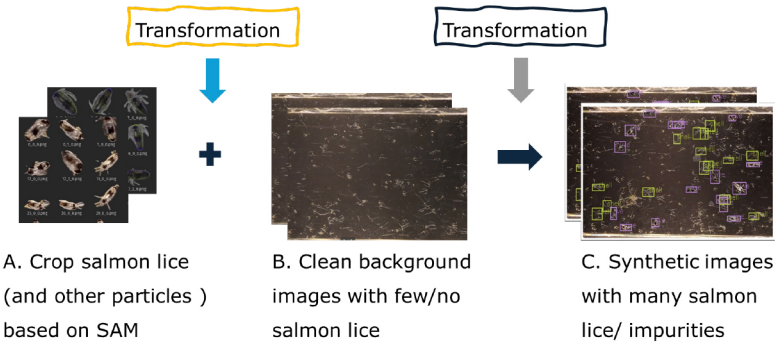


Fig. 1. Synthetic image generation process. Instances of salmon louse larvae and non-target particles (A) are overlaid onto clean background images (B) to produce synthetic images (C).

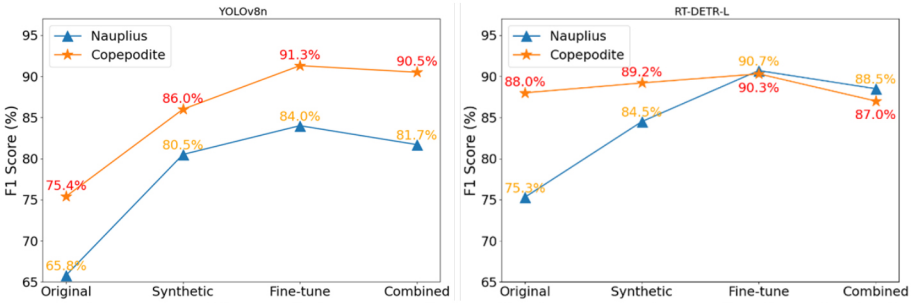


Fig 2. Performance of four training strategies on final test set using optimal synthetic data: Baseline, Synthetic-Only, Fine-Tuning, and Combined.

# EVALUATION OF A NOVEL BACTERIAL PROTEIN AS A SOYBEAN MEAL SUBSTITUTE IN RAINBOW TROUT (*Oncorhynchus mykiss*) DIETS WHILE CONSIDERING THE DILUTION EFFECT OF AN INERT BULK AGENT

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## Introduction

Soybean meal (SBM) is a predominant plant-based protein in fish feed due to its suitable protein content (~48%) and amino acid profile (Bai et al., 2022). However, previous studies indicated plant-based proteins are closely linked to land use-related emissions (Simon et al., 2024), and contain antinutritional factors and limited concentrations of methionine and lysine. *Clostridium autoethanogenum* protein (CAP) is a novel bacterial protein produced via the gaseous fermentation of one-carbon gases ($CO_2$, CO, and $H_2$) primarily sourced from industrial waste streams (Wang et al., 2023). Compared to SBM, CAP has a higher crude protein content (~80%) and a suitable essential amino acid profile, and its feasibility as a replacement for SBM has been evaluated in the diets of various fish species (Wu et al., 2024). However, replacing SBM with CAP in experimental diets may require the inclusion of an inert bulk agent, such as microcrystalline cellulose (MCC), which can reduce the nutrient density of the diet and consequently trigger compensatory feed intake in fish (Lekva et al., 2010). The aim of this study, therefore, is to evaluate the effects of replacing SBM with CAP on the growth performance of rainbow trout while accounting for the dilution effects on nutrient density.

## Materials and methods

The growth trial was conducted for 67 days in a recirculating aquaculture system (RAS) at Qinghai University, Qinghai, China. A total of 840 fish (initial body weight: 84.10 ± 0.08 g) were acclimated for three weeks to adapt to the experimental conditions. Three iso-nitrogenous and iso-lipidic experimental diets were formulated: (1) control: 20% SBM and 0% CAP; (2) CAPS6: 10% SBM and 6% CAP; and (3) CAPS12: 0% SBM and 12% CAP. The control diet contained similar composition and nutritional profile as commercial diets. Appropriate amounts of lysine, methionine, and threonine were added to balance the amino acid profile, and MCC was included as an inert filler to adjust all diets to a standardized total of 100%. Fish were hand-fed to apparent satiation twice daily (at 09:00 and 17:00), and daily feed intake was recorded. All water quality parameters were maintained within the optimal range to support fish growth. Growth performance parameters, including final body weight (FBW), feed intake (FI), feed conversion ratio (FCR), and weight gain rate (WGR), were calculated. To correct for the nutrient dilution effects, corrected feed intake (FIc) and corrected feed conversion ratio (FCRc) were also calculated.

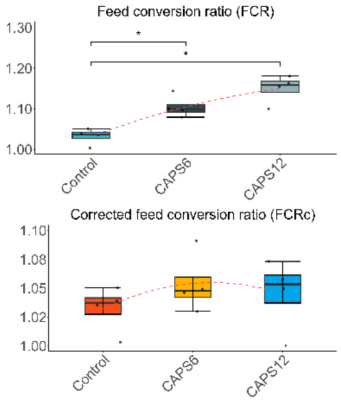


Figure 1. The feed conversion ratio of rainbow trout fed experimental diets. The red dashed line indicates the linear trend. The black dots indicate individual observations.

Figure 2. The corrected feed conversion ratio of rainbow trout fed experimental diets. The red dashed line indicates the linear trend. The black dots indicate individual observations.

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**Main results and discussions**

In present study, no significant differences in FBW, FI, or WGR were observed between CAP inclusion treatments and the control ($P > 0.05$). However, the FCR increased with rising CAP inclusion levels ($P < 0.05$) (Figure 1), whereas the FCRc remained comparable to the control ($P > 0.05$) (Figure 2). The current growth trial showed that CAP had no negative effects on rainbow trout growth. However, the inclusion of MCC may have introduced a nutrient dilution effect, thereby reducing the availability of digestible nutrients (Tran-Duy et al., 2008). As a result, fish may have responded by increasing feed consumption to compensate for the lower nutrient density (Costa et al., 2022). After correcting for nutrient dilution, FCRc in CAPS6 and CAPS12 was not significantly different from that of the control. Moreover, FIc showed a downward trend from the control to CAPS12, although the differences were not statistically significant.

**Conclusions**

This study indicated that completely replacing SBM with a novel bacterial protein did not significantly affect FBW, FI, or WGR. However, as the inert filler inclusion level increased, the nutrient density of the experimental diets was reduced, resulting in increased FCR as fish attempted to meet their nutritional requirements. Therefore, the nutrient dilution effects of inert fillers should be further investigated, especially in carnivorous species.

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# ENVIRONMENTAL FACTOR MANIPULATION IN RECIRCULATING AQUACULTURE SYSTEMS (RAS): EFFECTS OF LIGHT WAVELENGTH, SALINITY, AND TEMPERATURE ON FISH GROWTH PERFORMANCE

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Recirculating aquaculture systems (RAS) represent a crucial technological advancement in the aquaculture industry, with optimizing production efficiency. A key research priority remains to maximize and optimize the growth and reproduction of rearing fish species. Although environmental factors significantly influence fish growth, their systematic manipulation in RAS requires further investigation. This study examined the effects of specific environmental parameters on the growth performance of Malabar grouper (*Epinephelus malabaricus*), an economically important species in Asian markets. We evaluated the impacts of light wavelength, salinity, and temperature under controlled RAS conditions.

Under conditions of long-day photoperiod (light: dark = 14:10) and 21°C water temperature with red (peak at 632 nm), green (519 nm), and blue (465 nm) LED, and natural light, the specific growth rate (SGR) was significantly higher ($P < 0.05$) in fish exposed to blue LED light than to red LED light. In salinity trials conducted at 26°C, fish maintained at lower salinities (5 and 11 psu) demonstrated significantly higher SGR compared to those at higher salinities (22 and 34 psu) ($P < 0.05$). Temperature experiments revealed significantly enhanced fish SGR at 30°C compared to both 20 and 25°C ( < 0.05) under long-day photoperiodic conditions.

These findings demonstrate that the manipulation of environmental parameter can optimize fish growth in RAS operations. However, further research is needed to investigate potential interactive and synergistic effects among these environmental factors, which could provide additional insights for commercial aqua culture applications.

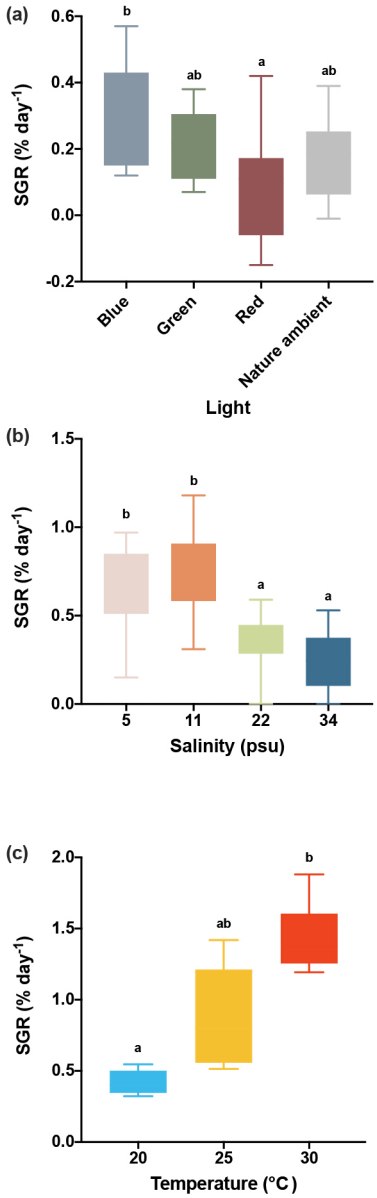


**Fig. 1. SGR of the Malabar grouper juveniles under different controlled RAS conditions: light wavelength, salinity, and water temperature.**

# META-LEARNING ENHANCED CNN TRANSFER LEARNING FOR INDIVIDUAL FISH IDENTIFICATION IN WILD POPULATIONS

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## Introduction

Accurate monitoring of individual fish within their natural settings is needed for ecological studies, conservation schemes, and sustainable fisheries management. A reliable, non-invasive identification system could provide valuable insights into health, and population dynamics with minimal effect on their ecological routines. However, no specific, standardized method currently exists for individual fish identification in wild and open-group populations. Developing such a farmework may seem impractical due to the visual similarity among individuals. Nevertheless, recent advances in artificial intelligence particularly in computer vision, convolutional neural networks (CNNs) for performing biometric tasks [1], transfer learning, and meta-learning offer a promising pathway toward automating this challenging task [2]. Herein, we present a prototype of a meta-learning-based biometric system developed to identify and match individual rainbow trout. The system was evaluated using data collected over a 70-day period from rivers across the Czech Republic. Preliminary results from the individual matching via developed system demonstrate promising outcomes, highlighting the potential of this approach for reliable open-group identification in natural water bodies.

## Materials and methods

### Dataset

We utilized an open-group dataset, which included still images of trout captured out of the water. All fish were consistently oriented to the same side for image capturing to ensure uniformity. Data collection was carried out over 10 sessions, and in each session, fish were carefully captured from the river, photographed, and subsequently released back into the sampling fishing site. At the conclusion of the data collection process, the dataset consisted of 535 images representing 425 individuals. Among these, 67 individuals were observed across multiple more than one time across sessions, providing valuable data for cross-session matching. Expert annotators manually labelled the dataset to match the same individuals across different sessions. Both individuals who appeared more than once and those captured only once were assigned unique class labels (ID of individuals). These labelled classes served as the basis for training and evaluating the proposed biometric identification system.

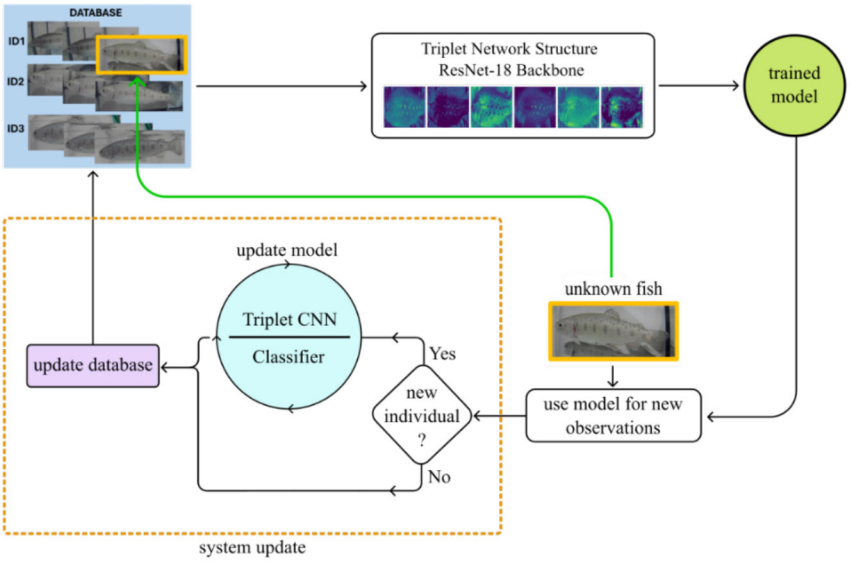


Figure1: Illustration of the proposed learning framework for open-group individual fish identification

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**Methodology**

The proposed system utilizes a triplet network with a ResNet-18 backbone for individual identification of trout. This method employs an incremental learning strategy, enabling the system to add new classes of individuals to update the model and database progressively. The model is trained from scratch on a dataset including five known individuals. After each training cycle, the model compares newly observed individuals with the existing database, adding into the individual's set if a match is found or assigning the image as a new class in case no match is detected. The model is continuously retrained after each cycle of database update with the transferred weights from the previous phase and improves the generalization ability effectively. A thresholding strategy is employed to balance the trade-off between confidence in matching and the risk of class fragmentation of the same individuals. This framework supports few-shot learning, enabling robust re-identification for limited observations.

**Results**

The preliminary results of the proposed method achieved a Top-1 identification accuracy of 80.3% at the final stage of the incremental learning process. A class prediction probability threshold of 0.33 yielded optimal performance, and few-shot evaluations on the dataset showed 84% accuracy with three or more observations per individual. The model also maintained strong identity grouping, with an Identity Consistency Rate of 89.7% and a Class Purity of 92.1%.

**Conclusion**

This study presented an incremental, meta-learning-based system for individual identification of open-group trout. The results demonstrate that the open-group identification of fish species can be achievable with a low number of observations per individual. By using a progressive learning strategy, the model achieved a satisfactory Identity Consistency Rate and Class Purity metrics, effectively demonstrating its ability to adapt to new individuals. This method also holds potential for practical applications in the field of aquaculture. Further development of the proposed system could provide a promising solution not only for ecological monitoring but also for advancing automated stock management in aquaculture.

**Acknowledgement**

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# BLUE MUSSELS: SINNERS OR SAINTS? GREENHOUSE GAS EMISSIONS FROM SHELL FORMATION IN THE NORTH ATLANTIC

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## Introduction

Farmed blue mussels (*Mytilus edulis*) are one of the animal-source foods with the lowest greenhouse gas emissions associated with their production, i.e. from material and energy use in spat collection to grow-out production (Gephart et al. 2021), mainly related to their independence from manufactured feeds. Feed often comprises the lion's share of farmgate emissions of farmed seafood (Bohnes et al. 2019). However, it has been suggested that the carbon dioxide released into the ocean during the calcification process of shells makes a non-trivial contribution to farmgate greenhouse gas emissions and excluding it may lead to an underestimation of total emissions (Ray et al. 2018). The Mussel Puzzle is a three-year project aiming to quantify the environmental impacts of different mussel production technologies across various locations using Life Cycle Assessment (LCA) while also assessing the potential for industry expansion and its related environmental trade-offs. Here, we quantify the carbon dioxide released over time from shell calcification in major mussel production areas in the North Atlantic.

## Methods

Carbon dioxide emissions from shell formation were calculated using the formula:
$CO_2$ Emissions = $\psi$ × Shell Mass × Percent $CaCO_3$ × Molecular Ratio

To do this, the buffering capacity ($\psi$) of seawater was calculated using the *psi* function within the *seacarb* package in R (Gattuso et al. 2024). This function estimates the seawater's buffering capacity under specified conditions. The $\psi$ calculation requires location-specific data on sea surface temperature, salinity, pH and the surface partial pressure of carbon dioxide in seawater ($spCO_2$). These data points were extracted from satellite data provided by the EU's Copernicus program. Carbon dioxide emissions from shell formation were then calculated on a monthly basis for the major mussel production countries in the North Atlantic, based on FAO production databases for the three most recent years available (2022 - 2024). Spain, as the most important European mussel producer, was included, although the main species produced is *M. galloproviancialis* rather than *M.edulis*.

## Results

Results show considerable variability in $CO_2$ emissions from shell formation across different sites, with Canada having the highest average emissions and Spain the lowest (Fig. 1). Emissions seem to be largely temperature-driven, as the ranking closely follows latitude. In particular, the northern latitudes show considerable seasonal variability, with higher emissions in the winter and lower in the summer, while seasonal variability in the southern latitudes is much smaller.

## Discussion

We show considerable variability in shell formation emissions across farming regions. In particular, northern latitudes show considerable temporal variation in emissions between summer and winter. It is important to note that not all of these emissions will reach the atmosphere and contribute to global warming, but it is difficult to determine exactly how much will. In our presentation, we will discuss this and relate the greenhouse gas emissions from shell formation to those generated by production processes, which have typically been included in greenhouse gas emission estimates. Whether mussels are sinners or saints, i.e. sources or sinks of carbon, also depends on what happens with shells after harvest and the time horizon in the assessment. We will discuss how to incorporate these findings into future greenhouse gas emissions accounting for farmed mussels.

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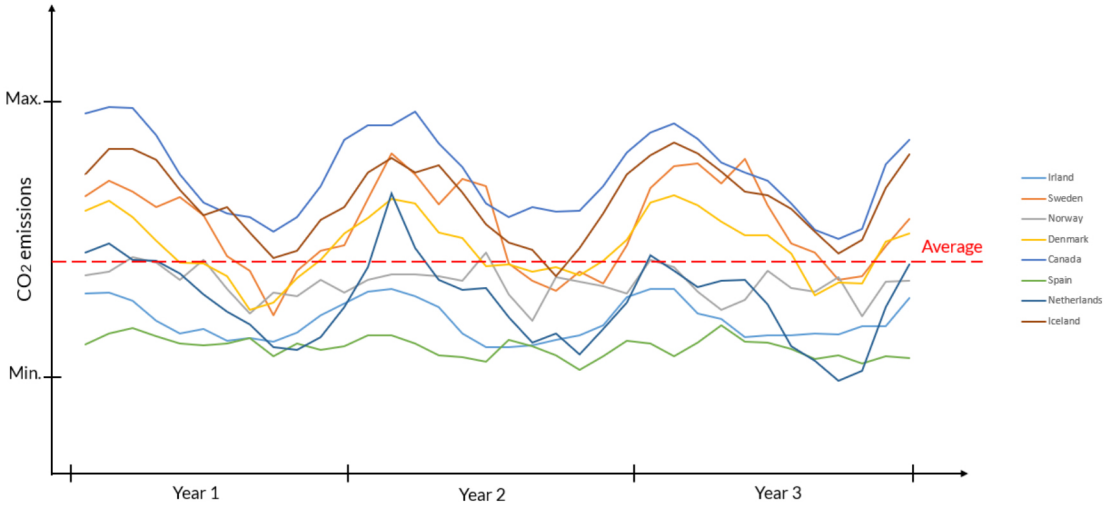


**Fig. 1** Relative carbon dioxide emissions from shell formation in coastal sites of countries bordering the North Atlantic, showing results relative to the average and highest values observed.

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# IMMUNE RESPONSE OF EUROPEAN SEABASS (*Dicentrarchus labrax*) VACCINATED WITH *Vibrio harveyi* AUTOGENOUS VACCINE

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**Introduction**

Adriatic aquaculture, like marine aquaculture across the Mediterranean, faces significant losses due to infections caused by *Vibrio harveyi*, an opportunistic bacterium. The disease has a seasonal pattern, typically occurring during the summer months when sea temperatures exceed 21 °C (Montánchez & Kaberdin, 2020). Affected fish exhibit symptoms such as lethargy, loss of appetite, depigmentation, erosions, haemorrhages at the base of the fins, skin ulceration, keratitis, corneal opacity, exophthalmia, and pale gills with haemorrhaging. Without treatment, mortality rates can be substantial (Zupičić et al., 2019). Outbreak management of *V. harveyi* relies heavily on the use of antibiotics. While good aquaculture practices can help mitigate losses to some extent, no commercial vaccines are currently available to prevent outbreaks. Moreover, only a few studies have evaluated the efficacy of autogenous vaccines in Mediterranean aquaculture—primarily for species such as sole (Arijo et al., 2005) and, more recently, flathead mullet (Strem et al., 2023). In contrast, several studies from Asian marine aquaculture report successful experimental vaccinations using different vaccine types against *V. harveyi* (Chin et al., 2019; Abu Nor, 2020; Zhang et al., 2021; Erfanmanesh et al., 2024).

The Interreg Italy–Croatia project MARINET aims to develop an effective autogenous vaccine against *V. harveyi* and to monitor the duration of immunity under real farming conditions.

*Material and methods*

Selected *Vibrio harveyi* isolates were cultivated in tryptic soy broth (TSB) supplemented with 2% NaCl to produce a starter culture for vaccine production. This starter culture was then fermented in a bioreactor to generate a formalin-inactivated whole-cell vaccine (FMC), reaching a final optical density ($OD_{600}$) of 3.6.

Upon arrival at the experimental facility, seabass fry was tested for the presence of *V. harveyi*. The experimental design included six groups (n = 10 per group), with a mean individual body weight of 10 grams, each tested in triplicate: Groups 1 & 2**:** Immersion-vaccinated for 1 minute in a 1:10 vaccine dilution (final concentration: $2.5 \times 10^8$ CFU/mL); Groups 3 & 4**:** Intraperitoneally (i.p.) vaccinated with 0.1 mL per fish (dose: $1.1 \times 10^9$ CFU/mL); Group 5: Mock-vaccinated by immersion in seawater for 1 minute; Group 6: Mock-vaccinated by i.p. injection of 0.1 mL phosphate-buffered saline (PBS) per fish.

Vaccine efficacy was assessed by measuring specific IgM titers in sera collected one-month post-vaccination using ELISA. Additionally, head kidney, spleen, and intestinal tissues were collected from six fish per group at 4 hours, 24 hours, and 10 days post-vaccination. Samples were preserved in RNAlater and stored frozen for analysis of gene expression related to immune response (Mitler et al., 2009).

After 36 days in experimental tanks, all fish were challenged with a virulent dose of *V. harveyi*. Ten days post-challenge, the Relative Percent Survival (RPS) was calculated following the method described by Amend (1981).
Vaccinated fish from the farm trial were also tested for specific IgM titres and expression of selected immune-related genes.

*Results*

Based on the Relative Percent Survival (RPS) results, intraperitoneal (i.p.) vaccination provided the highest level of protection (RPS = 100), although immersion vaccination also yielded satisfactory results (RPS = 67). These findings confirm that the autologous vaccine against vibriosis caused by *Vibrio harveyi* offers effective protection for juvenile European seabass.

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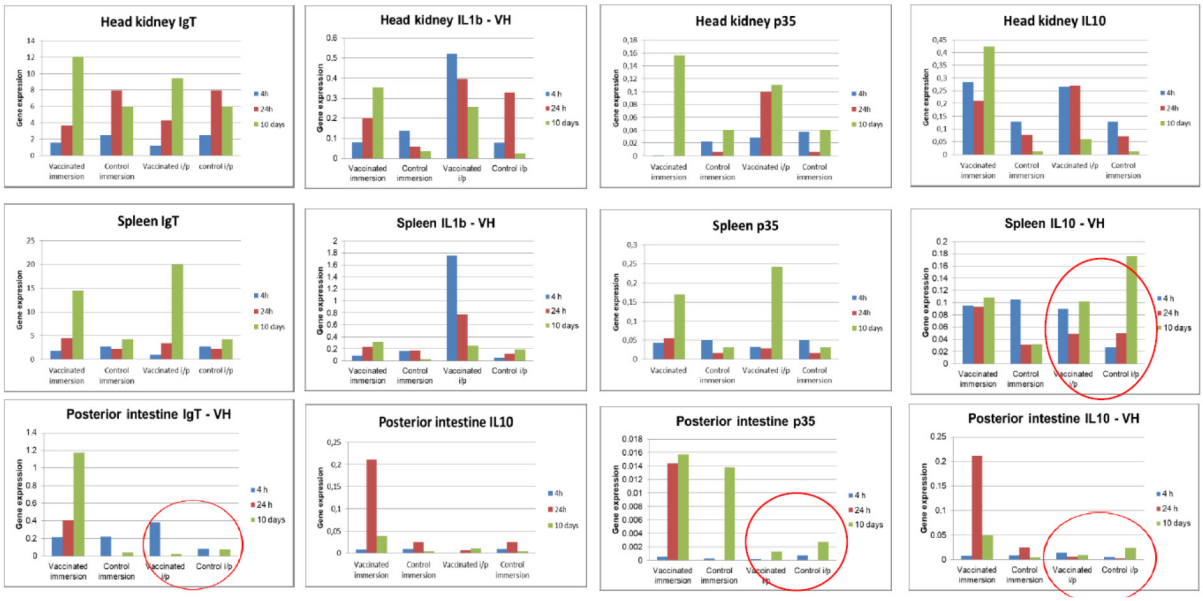


Analysis of immune gene expression showed upregulation of IgT in all examined organs for both vaccination routes, except in the intestine of i.p.-vaccinated fish, where no upregulation was observed. IL-1β expression was upregulated in the head kidney and spleen—and slightly in the posterior intestine—of immersion-vaccinated fish, while it was downregulated in the posterior intestine of i.p.-vaccinated fish. p35 was upregulated in the head kidney and slightly in the posterior intestine of immersion-vaccinated fish, but downregulated in the posterior intestine following i.p. vaccination. IL-10 was upregulated in the head kidney and spleen of immersion-vaccinated fish, but downregulated in both the spleen and posterior intestine of i.p.-vaccinated fish.

Sera were collected from laboratory-vaccinated fish 30 days post-vaccination and tested for specific IgM against *V. harveyi* bacterin using ELISA. Results showed a slightly higher IgM titre in immersion-vaccinated fish compared to mock-vaccinated controls (Fig. 9a). In contrast, i.p.-vaccinated fish exhibited significantly higher

IgM titres compared to the PBS-injected control group. In farm-vaccinated fish, specific anti-*V. harveyi* IgM titres were slightly elevated two and ten months after vaccination, both in fish vaccinated solely against *V. harveyi* and in those vaccinated with a combined vaccine (*V. harveyi* and routine *V. anguillarum*). Titres were higher at two months post-vaccination compared to ten months, and were elevated compared to the control group.

These findings provide a solid foundation for further optimization of vaccine formulation and support additional testing under field conditions to improve protection against *V. harveyi* in commercial aquaculture.

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# CONDITIONING OF THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*)

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Climate change threatens the sustainable cultivation of the Mediterranean mussel (*Mytilus galloprovincialis*) in the Adriatic Sea and affects its market quality. One measure of quality is the relative proportion of the edible part („meat“) measured by the condition index. In summer, at the time of highest market demand, high temperatures and fluctuations in water inflow from land can affect the reduced amount of food in nature and cause a drop in the condition index (CI=dry meat mass/shell mass) of cultured mussels. Time-limited shellfish farming in the recirculation system, along with controlled feeding, is a technological solution for mitigating the consequences of climate change. In this work, the influence of the size of the daily meal, of 3% ,4,5% and 6% dry matter of single-celled algae (*Tisocrisis lutea*) per gram of dry matter of meat on mussel CI, at three temperatures (12, 18 and 24°C), was evaluated. After acclimatization (7 days), after feeding for 14 days, and after starvation for 14 days, a sample of 15 individuals was taken from each pool to measure length, mass, mass of dried meat and calculate CI. The results obtained were tested by ANOVA Main Effects. The temperature had a significantly weaker effect than the size of a daily meal

# THE EFFECT OF INCLUDING FERMENTED AND HYDROLYZED *Rugulopteryx okamurae* IN THE DIET ON GROWTH AND FATTY ACID PROFILE OF EUROPEAN SEABASS

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**Introduction**

Since 2015, the expansion of the invasive macroalgae *Rugulopteryx okamurae* along the coastlines of Andalusia has resulted in the periodic accumulation of substantial quantities of algae on the region's beaches. In recent years, a variety of methods for the recovery of this algal biomass have been proposed, covering applications in various sectors, including cosmetics and pharmaceuticals, biogas production, the production of bioplastics and its use as compost (reviewed by Figueroa et al., 2025). Another particularly promising possibility for the valorisation of this biomass is its use as an ingredient and/or additive in animal feed, and more specifically in aquaculture, which could be of great interest due to the presence in algae of bioactive compounds with bactericidal, immunostimulant, and antioxidant properties (Barcellos et al., 2023; Cebrián-Lloret et al., 2024).). However, it has been demonstrated that pretreatment of the algal biomass is necessary, specifically through enzymatic hydrolysis and microbial fermentation, in order to improve its nutritional content and enhance nutrient bioavailability in the fish gut (Vizcaíno et al. 2024). The objective of the present study was to evaluate the influence of pretreated *R. okamurae* biomass dietary inclusion on European seabass (*Dicentrarchus labrax*) growth and fatty acid composition. In order to investigate this, European seabass juveniles were fed diets supplemented with pretreated *R. okamurae* biomass at levels of 5, 10, and 20% for a period of 110 days.

**Material and methods**

The algal biomass was collected from the coasts of the Strait of Gibraltar (Cádiz), subsequently washed and dried at 30°C for 12 hours, and subjected to a prior biotechnological treatment based on an enzymatic hydrolysis process followed by a fermentation process with microorganisms using a modification of the methodology described by Vizcaíno et al. (2024). In the feeding trial, four experimental isoprotein (44% dry matter [DM]) and isolipid (15% DM) diets were formulated. The control diet (CT) was prepared using a standard mixture of ingredients, without incorporation of algal biomass. The three other diets included processed biomass of *R. okamurae* at different inclusion levels: 5% (R5), 10% (R10), and 20% (R20), with wheat flour being mainly replaced. All experimental diets were developed by the Experimental Diets Unit of the University of Almería, based on previously validated formulations for juvenile European seabass according to Vizcaíno et al. (2024).

The *in vivo* feeding trial was conducted in Puerto de Santa María (Cádiz), at the facilities of the Institute for Agricultural and Fisheries Research and Training (IFAPA, El Toruño, Cádiz) of the Regional Government of Andalusia. The trial utilised 420 specimens, with an initial mean weight of 88.86 ± 1.45 g, provided by the aquaculture company CUPIBAR (Chiclana, Cádiz, Spain). Prior to the commencement of the feeding trial, the animals underwent a 15-day acclimatisation period, during which they were fed a commercial feed. The fish were then randomly distributed among 12 700-L tanks at a rate of 35 fish per tank, where they were fed the experimental diets for a period of 110 days. During the course of the trial, feeding was conducted using automatic feeders, with 20 daily rations being distributed over a 10-hour period (from 10:00 a.m. to 7:30 p.m.). Furthermore, feed quantities were modified on a weekly basis, with adjustments made based on a biomass estimate obtained from the most recent sampling data. These adjustments were made in accordance with feeding rates ranging from 1.5% to 1.8% of the total estimated weight. The experimental conditions were meticulously controlled and monitored to ensure optimal culture conditions. The temperature of the water was maintained at a constant level between 19°C and 20°C, and the photoperiod was set at 12 hours of darkness and 12 hours of light.

Total lipids (TL) were extracted using the chloroform: methanol method and gravimetrically quantified. For fatty acid methyl ester (FAME) determination, TL extracts were subjected to acid-catalyzed transmethylation and later they were separated and quantified by gas chromatography.

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**Results and discussion**

A significant decrease in the specific growth rate of juvenile sea bass was observed in fish fed diets containing 10% (SGR=0.74) and 20% (SGR=0.66) of pretreated *R. okamurae* biomass, compared to the CT treatment (SGR=0.80). No differences were detected in the vicerosomatic and perivisceral fat indices; however, a statistically significant decrease in the hepatosomatic index was observed in fish fed the R20 diet (HSI=1.49), respecting the CT group (HSI=2.22). This decline in the HSI was connected with a decrease in the lipids content of fish liver fed the R20 diet (59.0% DW) in comparison to the CT group (66.9% DW). Furthermore, a lower deposition of monounsaturated fatty acids (MUFA) and an increase in the relative levels of n-3 and n-6 polyunsaturated fatty acids (PUFA) were detected in liver in relation to the inclusion of RO in the diet. With regard to muscle lipid composition, no significant differences were observed in total lipids, saturated fatty acids (SAT), monounsaturated fatty acids (MUFA), and n-6 polyunsaturated fatty acids (PUFA) groups between experimental groups, although higher proportions of the polyunsaturated fatty acids arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were detected in fish fed the R20 diet.

The present study found that fish fed diets comprising more than 5% of pretreated *R. okamurae* biomass exhibited a significant decrease in growth, demonstrating a clear dose-dependent trend. This decline in growth performance could be attributed to the low digestibility of this macroalga for a carnivorous species such as sea bass (Cebrián-Lloret et al., 2024). Furthermore, a progressive decline in the hepatosomatic index and hepatic lipid reserves was observed with increasing algal biomass inclusion, suggesting a lower accumulation of energy reserves in the liver, possibly due to suboptimal absorption of essential nutrients. Finally, the stability of lipids and fatty acids composition of Sea bass muscle suggests that the use of *R. okamurae* in the diet does not compromise the quality of the final product.

**Acknowledgements**

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# RECOMBINANT FSH INDUCES STEROIDOGENESIS AND SPERMATOGONIAL PROLIFERATION IN IMMATURE FLATHEAD GREY MULLET (*Mugil cephalus*)

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## Introduction

In recent years, recombinant gonadotropins (rGths) have become increasingly important among technologies to overcome reproductive dysfunctions of cultured fish and to advance puberty. The administration of species-specific recombinant gonadotropins (rGths) for flathead grey mullet *Mugil cephalus*, was effective in overcoming the severe reproductive dysfunctions observed in this species under captive conditions, ultimately leading to the production of high-quality fertilised eggs (Ramos-Judez et al., 2022; Cerrud-Barría et al., 2025). This study aimed to analyse the effectiveness of a single injection of rFsh in inducing spermatogenesis in juvenile flathead grey mullet *Mugil cephalus* with recently differentiated testes and no signs of spermatogenic activity.

## Material and Methods

Grey mullet juveniles were caught from the wild population from Ebro river Delta and reared at IRTA (Ràpita, Spain) under controlled photoperiod. On 16 May 2024 (day 0 of the experiment) untreated fish (group C-0; N = 9) were sampled to assess the reproductive state on the day the other groups were treated. Treated fish (group rFsh-T; N = 10) were administered a single dose of 12 µg $kg^{-1}$ rFsh. Control fish (group C-7; N = 9) were given a single injection of 0.35 mL of saline solution. Fish from groups rFsh-T and C-7 were anaesthetised, sacrificed and sampled 7 days after treatment. Blood samples were taken and centrifuged to collect plasma for measuring 11-ketotestosterone (11-KT) concentration (EIA essay; Cayman Chemical Company, USA). Fish total length (TL, in cm), body mass (BM, in g) and gonad mass (GM, in g) were measured and gonadosomatic index (GSI) was calculated as 100 x GM/BM. Small testis slices were fixed in Bouin's solution and embedded in paraffin wax. Deparaffinized sections were stained with hematoxylin-eosin and proliferating spermatogonia were identified through the immunohistochemical detection of the proliferating cell nuclear antigen (PCNA; Santa Cruz Biotechnology Inc., Dallas, Texas).

## Results and Discussion

The GSI of the rFsh-T group was significantly higher than that of the C-0 group (0.06 ± 0.03 vs. 0.03 ± 0.02; $P < 0.05$), but not different from that of the C-7 group (0.06 ± 0.03 vs. 0.05 ± 0.01; $P = 0.34$). In both control groups, small seminiferous tubules were observed, which were predominantly composed of single undifferentiated type A (type $A_{und}$) spermatogonia (Fig. 1a). Among the ten fish treated with rFsh, six individuals displayed a testicular appearance similar to that of the control groups and were classified as non-responsive to rFsh treatment, whereas four rFsh-T individuals showed more advanced stages of germ cell development, including committed spermatogonia and spermatocytes (Fig. 1b), and were classified as responsive to rFsh treatment. No responsive fish were observed in control C-7 group. The diameter of the seminiferous tubules in responsive fish (42.5 ± 3.2 µm) was significantly larger than that of both control groups (C-0, 27.0 ± 3.8 µm; C-7, 28.4 ± 2.9 µm) and non-responsive rFsh-T individuals (32.9 ± 6.5 µm) ($P < 0.05$ in all comparisons). The rFsh-T responsive fish displayed a significant increase in proliferating committed spermatogonia compared with C-0 and C-7 ($P < 0.05$ in both comparisons) (Figs. 1c and 2a, b). The 11-KT plasma concentration was significantly higher ($P < 0.05$) in rFsh-T responsive fish compared to both control groups and non-responsive fish ($P < 0.05$) (Fig. 2c).

The results of the present study indicate that rFsh administration was effective in stimulating testis growth, proliferation of spermatogonia committed towards meiosis and 11-KT secretion in 40% of immature grey mullet with quiescent testes that did not show sign of spermatogenetic activity. This study suggests the potential suitability of the produced recombinant gonadotropin in advancing puberty in immature grey mullet reared under farming conditions.

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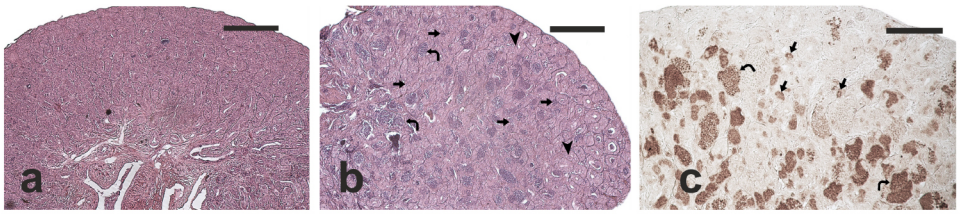


**Fig. 1.** Microphotographs of juvenile grey mullet testes. a) Testes from an untreated control fish (C-0 group). b) and c) Testis from a responsive rFsh-T fish. H-E staining in a) and b). Anti-PCNA immunostaining showing proliferating germ cells in brown in c). Magnification bars: 200 µm in (a) and (b), 100 µm in c). Arrow: spermatogonial cyst; arrowhead: single type $A_{und}$ spermatogonium; curved arrow: primary spermatocyte cyst.

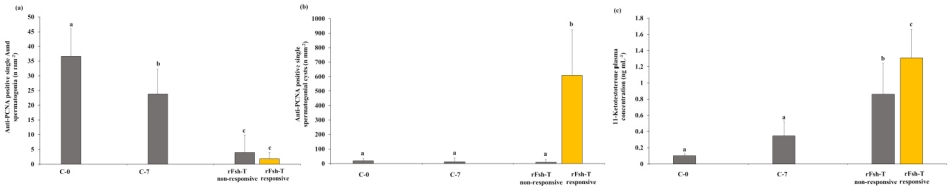


**Fig. 2.** Changes in mean (± sd) (a) anti-PCNA positive single type $A_{und}$ spermatogonia, (b) anti-PCNA positive spermatogonial cysts, and (c) 11-ketotestosterone (11-KT) plasma levels in juvenile grey mullet. C-0, untreated control group; C-7, control group treated with saline solution; rFsh-T, rFsh-treated group. Different letters represent statistically significant differences (ANOVA; $P < 0.05$).

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# IMPACT OF INDIVIDUAL QUICK FREEZING (IQF) ON NUTRITIONAL QUALITY OF THE OYSTER (*Ostrea edulis*)

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European flat oyster (*Ostrea edulis*) is a highly priced sea food product, while oysters from Mali Ston Bay (South Adriatic Sea) are labelled with designation of origin (PDO) due to interaction of environmental conditions and quality of the oysters. The aim of this work was to determine the changes of chemical composition in oysters preserved by using IQF technology, with a goal of freezing oyster during the period of the best quality (March/April).The oysters were sampled from the bivalve farms at Mali Ston area and the Krka River estuary in March 2023, when they are characterized by a high condition index, frozen in whole shell with liquid nitrogen at -80 °C, divided and stored at two temperatures: -18 °C and -60 °C for 3 months. No significant differences were observed in the increase in total volatile base nitrogen (TVB-N) with respect to freezing temperatures, while the pH value increased slightly only during the first month of storage, for both sites, respectively. Mass fraction of saturated fatty acids (SFA) in oyster samples from both sites increased slightly during storage or had values close to the initial ones at both temperatures. In both types of oysters and at both storage temperatures, the mass fraction of polyunsaturated α-linolenic fatty acid (C18:2 *cis*) decreased, while the proportions of α-linolenic (C18:3n3) and *cis*-13,16-docosadienoic acid (C22:2) increased during storage. The greatest change in the proportions of polyunsaturated fatty acids occurred with the decrease in the proportion of linoleic acid in Ston oysters, mostly during the first month at both temperatures. Compared to the initial samples, a higher mass fraction of monounsaturated fatty acids (MUFA), namely palmitoleic acid (C16:1) was quantified and the mass fraction of oleic acid (C18:1 cis) was quantified in a lower mass fraction, respectively. The results obtained show that the IQF technology is a good method for preserving oysters and that the storage temperatures of -18 °C and -60 °C do not affect their quality and remain acceptable to consumers.

# ADDENDUM

## EGNOS FOR AQUACULTURE OPERATIONS

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Aquaculture is evolving towards increasingly technological and sustainable practices, requiring innovative solutions. Among these innovations, the European Geostationary Navigation Overlay Service (EGNOS), Europe's regional Satellite-Based Augmentation System (SBAS), provides enhanced Global Positioning System (GPS) performance by improving accuracy, availability, and reliability. EGNOS is freely accessible and offers significant benefits for multiple aquaculture applications.

Here is presented the potential of EGNOS to support key operational areas in aquaculture. First, support in geofencing/marking of aquaculture zones and elements: precise delimitation of aquaculture zones is essential for safety, environmental monitoring, and efficient operations. EGNOS enables reliable geofencing and management of aquaculture areas. Second, support in vessel navigation for aquaculture: efficient vessel navigation is necessary in coastal aquaculture for transport, inspection, and maintenance activities. Third, support in autonomous vehicles—both surface (USVs) and underwater (AUVs, ROVs)—is increasingly deployed for inspection, monitoring, and maintenance. Their efficiency and safety rely heavily on high-accuracy positioning and trusted navigation data, which can be effectively provided by EGNOS.

The integration of EGNOS into aquaculture practices can enable smarter farm management, improve resource allocation, and reduce operational costs. Additionally, by supporting the adoption of intelligent feeding systems, EGNOS contributes to more sustainable aquaculture production. In summary, EGNOS stands out as a free, robust, and accessible solution to enhance positioning and navigation for aquaculture, paving the way for safer operations and the deployment of innovative technologies in the sector.

# SUSTAINABLE SOLID REMOVAL WITH ORGANIC COAGULANTS: OPPORTUNITIES FOR DOWSTREAM USE IN INTEGRATED AQUACULTURE

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## Introduction

Flocculation and coagulation are critical processes in aquaculture systems for removing suspended solids, organic matter, and colloidal particles. However, commonly used inorganic and synthetic coagulants present several drawbacks, such as optimal pH levels that fall outside the range typically achievable in aquaculture environments, sensitivity to the system's pH, or the toxicity of the substances and their residues (Ebeling et al., 2003). As a result, organic and bio-based flocculants/coagulants—such as those derived from starch or chitosan—are emerging as promising alternatives. Despite their higher costs and price volatility, which currently limit broader adoption (Chik et al., 2024), these organic agents offer the advantage of being biodegradable. The resulting sludge may therefore hold additional value for circular processing applications, including biogas production or microbial stabilization, with the potential to repurpose the liquid fraction or solid residues as fertilizer. The primary objective of this study was to evaluate the performance of selected inorganic and organic coagulants for solids management in a recirculating aquaculture system (RAS).

## Materials and methods

To assess the effect of flocculants and coagulants, jar tests were performed. The raw water was sourced from the backwash collection tank of a drum filter installed in a RAS with African catfish (for more details see Tellbüscher et al., 2025). The initial characteristics of the water were determined as follows: sludge volume was measured using Imhoff cones in triplicate, and a 100 mL supernatant sample was collected for analysis of total organic carbon (TOC), total nitrogen (TN), and orthophosphate ($P\text{-}PO_4^{3-}$). The pH and temperature were recorded immediately after sampling. Sludge dry matter content was determined using a moisture analyzer. 500 mL of raw water was transferred into each of four beakers using a graduated cylinder, with three beakers receiving the flocculant/coagulant treatment and one serving as a control. The equivalent volume of liquid to be replaced by the flocculant/coagulant was removed prior to addition. The beakers were placed in a jar test device with paddles positioned approximately at mid-water level. The samples were stirred at 200 rpm for one minute (mixing phase), followed by 20 rpm for 30 min (agglomeration phase). Subsequently, 100 mL of supernatant was collected from the middle of the water column using a 100 mL syringe and transferred into a 150 mL beaker. The pH was measured immediately, and residual turbidity was determined. Additional 50 mL of supernatant was filtered through a CA syringe filter, acidified with 200 µL of concentrated HCl, and stored at 4°C until further analysis. The results are presented as a percentage change to the initial sample parameters.

## Results

The percentage changes of the TOC, $P\text{-}PO_4^{3-}$ and FNU after the addition of different dosages of the tested coagulation agents are shown in Figure 1. All the organic and bio-based coagulants increased the TOC in the water, while the TOC concentrations increased with the coagulant dose. On the other hand, iron- and alluminium based coagulatns decreased the TOC concentrations. Phosphates were unaffected by the organic and bio-based coagulants compared to the synthetic ones wich decreased the concentrations almost 80-90% even with the lowest dosage tested (50 mg $L^{-1}$). No effect was found in turbidity (FNU) either among the tested coagulants/flocculants neithger among their different dosages.

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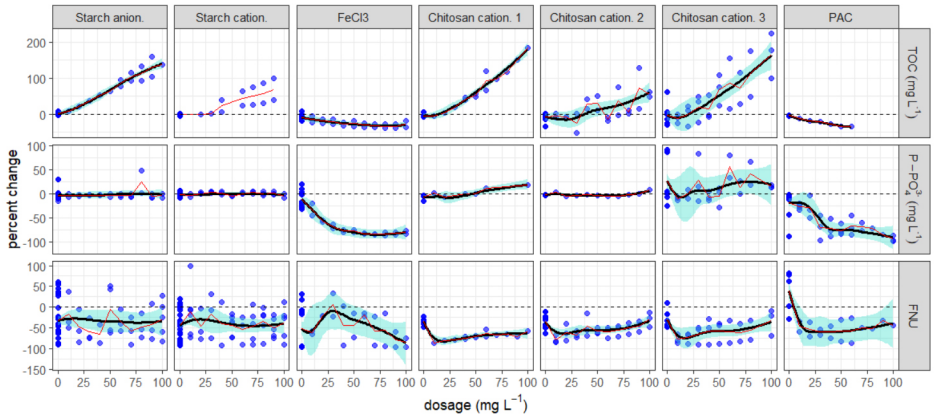


Figure 1. Percentage changes of the TOC, P-PO43- and FNU after the addition of different dosages of the tested coagulation agents.

## Discussion and conclusion

Ebeling et al., (2023) documented 89 and 93% phosphate removal efficiency for $AlCl_3$ and $FeCl_3$, respectively. Similar phosphate removal was achieved with the $FeCl_3$ and PAC in the present study while chitosan- and starch-based coagulants were not effective in phosphate removal. The low phosphorus removal was because the charge density of organic- and bio-based coagulants was low. In case of $FeCl_3$, there are other mechanisms such as formation of precipitates. A similar process has been described for alum. Chik et al. (2024) documented 90±0.72% turbidity removal using 10 mg $L^{-1}$ chitosan in the aquaculture wastewater. Similar result was achieved with chitosan-based coagullats while the starch-based ones were inconsistend in the turbidity removal. The different results might be due to differences in deacetylation degree. The starch-based coagulants are most likely effective at different condition (e.g. stirring).

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# ELECTROCHEMICAL STIMULATION OF CORAL GROWTH IN *Echinophyllia lamellosa* UNDER CONTROLLED AQUARIUM CONDITIONS

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## Introduction

Coral reef degradation is accelerating worldwide, demanding novel restoration strategies. Electrochemical mineral accretion through seawater electrolysis has emerged as a promising technique to promote calcium carbonate deposition, thereby providing a substrate for coral growth. However, fundamental studies under controlled laboratory conditions remain scarce, with most previous research limited to in situ trials.

## Materials and methods

An integrated 1400 L reef aquarium system was employed, comprising two 600 L frag tanks and two 100 L cubic tanks with independent filtration, circulation, and thermoregulation.

The seawater is prepared using deionized water as solvent and synthetic sea-salt formulated specifically for coral cultivation. Physicochemical and Major ions are monitored to encompass factors influencing the health of the marine ecosystem.

Conductive carbon paper cathodes were polarized to induce hydrogen evolution and oxygen reduction, generating hydroxyl ions that promoted local mineral deposition of aragonite and brucite (Figure 1). *Echinophyllia lamellosa* fragments were placed directly on preconditioned cathodes, while controls were placed on inert plastic grids (Figure 2).

## Results

Preliminary studies covering nearly 4 weeks, the growth of the different individuals on the cathode polarized electrode and on the control, grid was determined.

Figure 5 shows the variation of the area measured after this period for both sets of fragments. It is observed that in the electrochemical system the increase in area was around 10%, while in the individuals located on the control grid the growth was roughly half (increase in area of around 5%).

## Discussion and conclusions

Preliminary results confirm that electrochemical stimulation promotes coral growth under controlled aquarium conditions, supporting its potential as a complementary tool for reef restoration. Further optimization of electrolysis parameters and long-term trials are ongoing to evaluate species-specific responses and ecological feasibility.

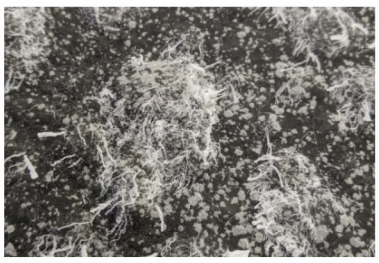

Fig. 1: Mineral deposition in cathode.

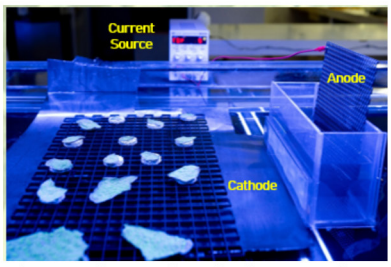


Fig. 2: Electrochemical set-up for coral growth

To analyze coral growth, a series of photographs were taken weekly. These images were classified chronologically and analyzed with ImageJ software, which allowed the measurement of coral area (Figure 3 and 4).

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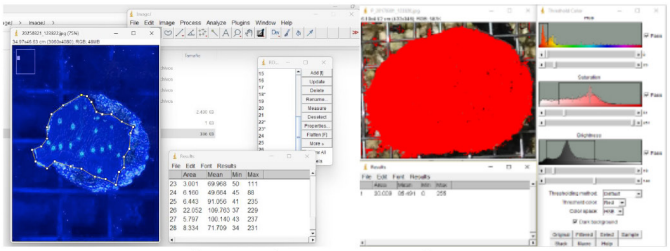

Fig 3 and 4: ImageJ software

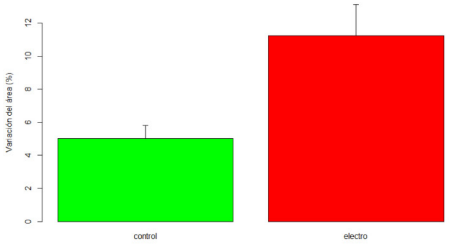


Fig. 5: Measured area variation between control and treated corals

# GALILEO SERVICES FOR AQUACULTURE: ACCURATE, RESILIENT, SUSTAINABLE AND SAFER OPERATIONS

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**Introduction**
Galileo is the European Global Navigation Satellite System (GNSS), under civil control, that provides Positioning, Navigation and Timing (PNT). As connected aquaculture operations expand offshore and face growing GNSS threats such as jamming and spoofing, there is an increasing need for precise and resilient positioning and navigation solutions. Galileo provides free services that enhance operational efficiency, sustainability, and safety for aquaculture stakeholders worldwide.

**Galileo Services and Implementation**
In addition to the Open Service (OS), Galileo's portfolio includes the Open Service Navigation Message Authentication (OS-NMA), the High Accuracy Service (HAS) and the Search and Rescue Service (SAR). These differentiators have potential to support aquaculture activities such as site selection, farm zoning, offshore structure deployment, autonomous monitoring, route optimization and product traceability among others.

- High Accuracy Service (HAS): Provides free centimetre-level accuracy (<20 cm horizontal, <40 cm vertical) using a Precise Point Positioning (PPP) algorithm. This enables eco-friendly site location with reduced habitat damage, and optimized routing for inspections.

-Open Service Navigation Message Authentication (OS-NMA): Enhances resilience by protecting against spoofing and jamming incidents, ensuring GNSS signal authenticity for product traceability and autonomous operations.

-Search and Rescue Service / Return Link Service (SAR/RLS): Supports maritime safety by locating people and vessels in distress and confirming reception of emergency alerts.

- Implementation: Most GNSS devices already support Galileo OS. Integration of Galileo differentiators for aquaculture activities can be straightforward and cost-effective. Free advice and support are provided by the European Union Agency for the Space Program (EUSPA) and the European GNSS Service Centre (GSC).

**Conclusion**
Galileo delivers free, accurate, and resilient positioning services that directly support the sustainable growth of aquaculture. By enabling precision farming, strengthening digital trust and enhancing safety at sea Galileo contributes to efficient, ecological and safer aquaculture operations in Europe and worldwide.

# REDUCTION OF SKELETAL DEFORMITIES IN SENEGALESE SOLE BY DIETARY SUPPLEMENTATION WITH *Skeletonema costatum*

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## Introduction

Skeletal malformations in farmed fish affect productivity, growth, reproduction, survival and increase susceptibility to stress and pathogens, but they also downgrade the quality of the produced fish, preventing it from being marketed (Boglione 2013). These deformations have a multifactorial origin and constitute one of the main constraints in fish farming, requiring strategies to reduce and mitigate their incidence. Species produced in southern Europe, like sea bream (Sparus aurata), sea bass (Dicentrachus labrax) or Senegalese sole (Solea senegalensis) often reach levels above 50% of malformations during larval and juvenile stages, and the industry can benefit greatly from technical improvements that can promote a reduction in the incidence of these malformations.

Nutritional imbalances affecting bone development are one of the main factors affecting the incidence of skeletal deformities, which are acknowledged to constitute one of the major constraints in the production of high-quality fish. It has recently been shown that the use of microalgae like *Skeletonema costatum* as supplement in fish feeds produces beneficial effects, by improving fish quality and reduction of skeletal malformations in *S. aurata* and *Danio rerio* (Carletti 2023). *S.costatum* is a known source of antioxidants and essential nutrients such as vitamins, minerals, and polyunsaturated fatty acids, and is capable of producing numerous bioactive molecules with nutraceutical and pro-osteogenic properties (Carletti 2023).

Our objective was to determine the effects of a dietary supplementation with *S. costatum* during larval and early juvenile stages of *Solea senegalensis* over the growth and survival performances and on the development of skeletal deformities.

## Materials and Methods

Solea senegalensis larvae at 13 dph were distributed into 6 flat bottom tanks with 0.1m$^2$ at a density of 300 larvae per tank. The tanks were randomly assigned in triplicates to two dietary treatment groups: control group fed *Artemia* enriched with *Nannochloropsis* sp.; and an experimental group fed *Artemia* enriched with a blend of *Nannochloropsis* (75%) and *Skeletonema costatum* (25%).

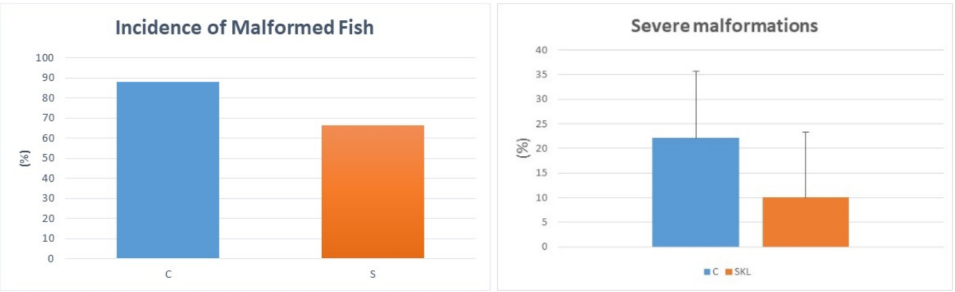


Figure 1- Incidence of skeletal malformations and incidence of severe malformations in Senegalese sole fed control diet (C) and *S. costatum* supplemented diet (S).

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A large batch of Artemia was enriched at the beginning of the experiment with either *Nannochloropsis* sp. alone or a mix of *Nannochloropsis sp.* (75%) and *Skeletonema costatum* (25%). After the enrichment the nauplii were collected, counted and frozen in portions of 20k. Fish were fed 3 times $day^{-1}$ at 4% BW and the portions were adjusted daily to sustain growth. Mortality was registered daily. Water temperature was maintained at 20.5 ± 1ºC, D.O. 95-100%, and salinity 36 ± 1ppt. Growth in length and dry weight was assessed at 23 dph and 33 dph. At the end of the trial (33dph) a sample of 30-35 specimens per replicate was collected to determine skeletal deformities and mineralization and 5 fish per replicate were collected for determining oxidative status.

**Results**

At the end of the trial the larvae from the treatment group presented a larger length (10,86 vs 9,46 mm) and weight (3,31 vs 2,59 mg) compared to the control and

A marked reduction of 20% was observed on the incidence of malformations on the group fed Skeletonema costatum supplemented diet and a high reduction on the number of fish presenting severe deformities was also observed.

In addition, the number of deformities observed per fish was reduced from 4,2 in control to 3 in the treatment group. The incidence of malformations affecting the caudal and pre-hemal vertebrae were also reduced, translating into fish with greater quality and less prone to development of vertebral curvatures like lordosis-kyphosis and scoliosis, that significantly deteriorate fish quality.

**Discussion**

In this study we tested the use of a diet composed of artemia enriched with 25% Skeletonema costatum, and evaluated the effects on growth, survival, and on the frequency and severity of skeletal malformations in *Solea senegalensis* larvae, a species with high commercial value and very susceptible to developing deformities during larval stages (Gavaia et al., 2002). Our results showed that S. costatum induces a protective effect of on the development of skeletal deformities during early stages, with a reduction on the number, charge and severity of skeletal malformations. In addition, the larvae treated with the S. costatum enriched diet presented a better growth performance, with greater length and weight at the end of the experimental period. The fact Skeletonema costatum presents a nutritional profile rich in fatty acids, antioxidants, minerals and biocatives (Hemaiswarya et al., 2011, Carletti et al., 2023) indicates that it is providing a more balanced nutritional profile to the diet during larval growth, sustaining a more harmonious growth and improving the quality of the produced specimens.

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**Aknowledgements**

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# BLUE MUSSEL *(Mytilus edulis)* AS A SUSTAINABLE BIOFACTORY

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The aim of our work is to use the biosynthetic potential of blue mussels as a natural biofactory for the formation of tailored valuable biocompounds by varying the genotype, farming conditions and applying stress interventions. We aim to explore the biochemical potential by using a broad range of cutting-edge analytical techniques. We aim to identify novel compounds and novel functions by fundamental characterization of physicochemical and technological properties relevant for food and non-food applications. We are aiming for tailored compounds by using fractionation and isolation strategies that follow the requirements of the target compounds as well as the principles of bioraffination.

**Background**
Amongst marine organisms, mussels are very unique. During their lifespan, they undergo dramatic changes leading to strong variation in the metabolism of the organism. After its planktonic stage the organisms transform into a sessile mussel and grows tremendously within the first months until the mussels reach a plateau phase during the colder winter months and start to prepare for propagation in the next spring using up stored lipids and proteins. Blue mussels (*Mytilus edulis trossulus complex*), keystone species in the Baltic Sea ecosystem, represent one of the major aquaculture shellfish species. Although their popularity as sea food has increased over the last years because of their protein-, omega-3 long chain polyunsaturated fatty acids (LC-PUFA) and iodine content their potential as source for tailored biochemicals, in particular functional proteins, has been rarely explored. At the same time blue mussels also dominate the production of waste material in the shellfish sector, as only intact mussels of a certain age and size are commercially used as sea food. Shells, undersized, broken and those used for water engineering purposes are discarded.. Further, the metabolism of organism underlies external factors such as seasonal changes, feed source,and water depth. Finally, the mussel shells is a resource of biominerals comprised of two polymorphs of CaCO3, calcite and aragonite (mother of pearl). To date, some applications of shells as use for chicken feed, as a low-value material for concrete production or for the treatment of polluted soil and waters are described.

**Selected results**
Blue mussels were produced by Kiel Marine Farm in long-line farming systems and provided to CAU. Subsequently, Mussels were dissected into their components (shell and mussel meat). The mussel meat was fractionated to isolate proteins and lipids. Proteins. In order to characterize the protein profile of mussels SDS-Page electrophoreses were conducted. Using a multistep purification procedure we aimed for highly valuable myofibrillar proteins. Sarcosplamatic proteins together with other water soluble proteins were collected for further characterization. A special aspect is that black colouring occurs during the fractionation of mussel proteins. For the analysis of the physico-chemical and technological properties of mussel proteins a wide range of methods has been established (FT-IR, fluorescence spectroscopy, zeta-potential, hydrodynamic diameter). Fundamental properties such as solubility and surface charge (zeta potential) at different pH values have been examined for the myofibrillar fraction to give indications for the range of application. E.g. increased water binding capacity can be expected above a pH of 6. Stabilization of emulsions increases with amount (negative of positive value) of the Zeta-potential indicating repulsive forces above and below a pH value of 4.

To make wide use of mussel meat, a product is required with low taste, flavor and light color but high nativity of the functional proteins. This was conducted by applying several non-thermal procedures resulting in a meat homogenate. Subsequently, prototyües of innovative mussel food products were developed and underwent sensory evaluation. Furthermore, the utilisation potential of undersized mussels was contemplated. A comparison of cohorts revealed a significant variation in the meat-shell ratio, which was found to be dependent on age. The analysis revealed that mussels one year old contained 33% mussel meat, while those that were three years old contained 22%. This observation is of great significance for the economic production of biocomponents in musselsn this study, the meat of undersized mussels was utilised in the development of pet food products exhibiting a high protein value and quality.

The mussel shell resulted as by-product, we developed powdered fractions to be used in non-food application (e.g. cosmetics and filtermaterials). In addition, the blue pigments found in mussel shelll are of great interest due to their rarity in natural sources.

# FROM FARMED TO WILD: ISOTOPIC MARKERS FOR THE DETECTION OF ESCAPEES AND THE ASSESSMENT OF TROPHIC POSITION AND POTENTIAL NICHE OVERLAP IN GILTHEAD SEABREAM (*Sparus aurata*), EUROPEAN SEABASS (*Dicentrarchus labrax*) AND MEAGRE (*Argyrosomus regius*) IN THE WESTERN MEDITERRANEAN

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## Introduction

Marine aquaculture is expanding rapidly to meet global seafood demand (FAO, 2024), but escape events pose ecological and management challenges. Up to 9.6% of *Sparus aurata* and 0.6% of *Dicentrarchus labrax* annual landings in the Mediterranean originate from escapes (Toledo-Guedes *et al*., 2024). However, extreme storms like Gloria (2020) can cause total stock loss. Escapees interact ecologically with wild fish (Arechavala-Lopez *et al*., 2017) and are often sold as wild, violating EU traceability rules (Jackson *et al*., 2015). The GLORIA-TOOLS project, building on the Fundación Biodiversidad GLORiA initiative, develops tools for escapee detection, including stable isotope analysis (SIA) of muscle tissue.

## Materials and methods

A total of 102 muscle samples of seabream, seabass and meagre were collected from aquaculture sites and fish markets in Alicante and Murcia (Spain). Stable isotope analysis was performed at the University of A Coruña (SAI) using a MAT253 IRMS (Thermo Finnigan) with FlashEA1112 elemental analyzer. Tin-encapsulated samples were combusted at 1020 °C in a quartz column containing chromium oxide and silvered cobaltous/cobaltic oxide. Excess oxygen and nitrogen oxides were reduced in a copper column at 650 °C, and the resulting $N_2$ and $CO_2$ gases were separated by gas chromatography before entering the IRMS. $\delta^{13}C$ values were lipid-corrected (Post *et al*., 2007), and $\delta^{13}C/\delta^{15}N$ data were used in PERMANOVA to test for differences between wild, escaped, and farmed groups. Results were visualized in $\delta^{15}N$ vs. corrected $\delta^{13}C$ scatterplots.

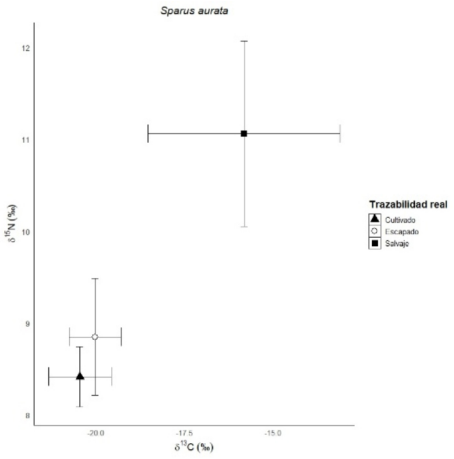


Figure 1. $\delta^{15}N$ vs. corrected $\delta^{13}C$ seabream (Sparus aurata) scatterplot.

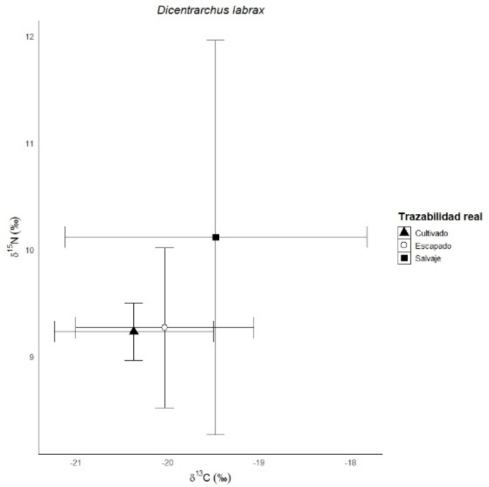


Figure 2. $\delta^{15}N$ vs. corrected $\delta^{13}C$ seabass (*Dicentrarchus labrax*) scatterplot.

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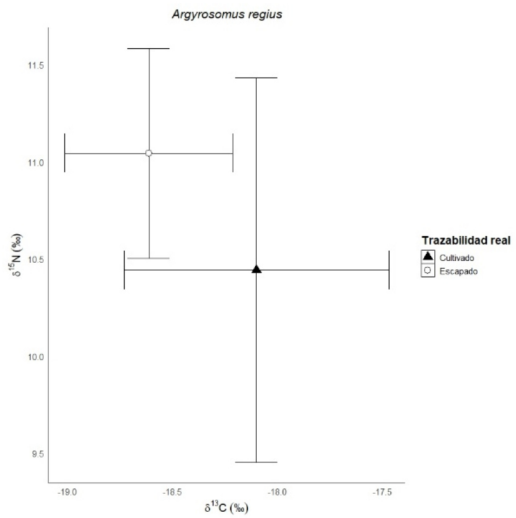


Figure 3. $\delta^{15}N$ vs. corrected $\delta^{13}C$ meagre (*Argyrosumus regius*) scatterplot.

**Results**

Sea bream showed clear, significant isotopic separation (Fig. 1): farmed fish had lower $\delta^{15}N$ and $\delta^{13}C$, wild fish were enriched, and escapees clustered in between.

Sea bass showed no apparent isotopic distinction among groups (Figure 2), which resulted in non-significant results.

Meagre lacked wild references, but $\delta^{15}N/\delta^{13}C$ scatterplots (Fig. 3) showed visual separation between farmed and escapees, with only marginal statistical differences.

**Discussion**

Sea bream showed clear isotopic separation, likely due to its omnivorous diet and plant-based feed, distinct from wild sources. In contrast, sea bass's piscivorous habits and animal-protein-rich feeds lead to overlap between wild and farmed signatures. Meagre lacks wild references, escapees are usually captured near cages shortly after escaping, still feeding on pellets, and then disperse to deeper habitats. These results show that SIA works best in species with distinct diets and lower trophic levels. In carnivorous fish, feed similarity reduces resolution, and complementary tools (e.g., stomach content or genetics) may be required.

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# GLORIA-TOOLS: GLOBAL CHANGE RESILIENCE IN AQUACULTURE – TOOLS FOR LONG-TERM SUSTAINABILITY

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GLORiA-TOOLS aims to develop and transfer tools and technologies reaching Technology Readiness Levels (TRL) 4–6, with the potential to enhance the environmental and socioeconomic sustainability of offshore fish farming under global change, particularly addressing the impact of fish escapes on marine ecosystems and consumer safety. Building on the outcomes of previous GLORiA and GLORiA2 projects, which identified key escape-related risks and suitable traceability markers, GLORiA-TOOLS focuses on delivering functional prototypes and decision-support systems to support escape management from aquaculture facilities.

The project is structured around six specific objectives: (1) to create a web-integrated GIS-based tool for spatiotemporal modelling and prediction of escape risk and its environmental impact; (2) to assess the socioeconomic consequences of escapes and provide training and decision-support resources to stakeholders; (3) to design a biosensor prototype based on traceability biomarkers for fish origin identification; (4) to identify and validate genetic traceability markers for farmed and wild fish; (5) to develop an artificial vision system for fish recognition in realistic settings; and (6) to promote user-driven evaluation and prepare prototypes for real-world deployment.

GLORiA-TOOLS will provide beta-phase tools for environmental modelling, genetic analysis, biosensing and machine learning, all validated through interdisciplinary research and prior field experience. These tools will contribute to informed management, policy development, and traceability enforcement across the aquaculture sector.